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PSYCHOPHYSIOLOGICAL RESEARCH METHODS[1]

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In a paper published 25 years ago, Adolf Meyer (1) stated that psychobiology begins not with a mind *and* a body nor with elements, but investigates the function of biologically organized units. As my classmates remember, he liked to contrast the activity of what he described as more or less detachable organs such as the heart, stomach or brain, with the activity of these same parts when integrated with the rest of a living person. In the years since our graduation, the emphasis of biochemistry has shifted from the analysis of the chemical constituents of organs and tissues to the subtle mechanisms by which the body transforms one substance to another. Recent work at the University of Wisconsin (2) has suggested, however, that even intracellular enzymes are built into specific structures, that their function depends to a large extent on organization, and that the enzyme activity of composite systems may not always be demonstrated as the sum of the individual enzymes contained therein. Thus the concepts of integration and organization seem to apply not only to the total organism but also to an ultramicroscopic part.

The fact that psychobiology and enzymology make use of certain similar principles reassures those of us who left the practice of general medicine to enter the field of psychiatry but still like to feel that we have maintained our allegiance to the concepts of medical science. In a recent lecture at the Massachusetts Institute of Technology on the subject of atoms and human knowledge, Nils Bohr (3) reminded his audience that the locating of an object in a limited space-time domain involves, according to quantum mechanics, an exchange of momentum and energy between instrument and object, which is the greater the smaller the domain chosen. It has therefore been of the utmost importance, as he said, to investigate the extent to which the interaction entailed in observation can be considered separately in the description of the phenomena. In fact, as Bohr points out, the whole experimental arrangement must be taken into account in a well defined description of the phenomena. This corresponds, of course, to the emphasis of psychoanalysis on the micro-

[1] Delivered at Biannual Meeting of the Johns Hopkins Medical and Surgical Association, Saturday, February 28, 1959, as part of the clinical program given by the 25th Anniversary Classes.

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scopic investigation of the conscious and particularly the unconscious interaction of the patient with the observing analyst referred to as the phenomena of the transference and of the counter-transference. Thus, neither the atomic physicist nor the psychoanalyst can keep up the fiction of an Olympian scientist who merely records what he observes without any regard for the interaction of the observing instrument with the object of observation.

Although predictability remains a highly desirable ideal for the scientific validation of hypotheses in physics or in psychology, the nature of the data under investigation does not permit the useful application of the simple type of experiment in which all of the variables except the one under study can be satisfactorily controlled. Dr. Meyer used to counsel us to be satisfied with a more modest observation of what he referred to as experiments of nature. Those of us who work with complex data do the best we can under the circumstances but have learned to avoid oversimplification of the essentially multivariable situation.

Psychophysiological research, after a period of disillusionment with incompletely successful attempts to demonstrate definite causes for structural diseases in specific psychological conflicts (4), has now embarked on what seems like a more promising attempt to establish correlations between disturbances in relatively constant biological and psychological rhythms, equilibria, and defensive balances (5). We are in the age of teams (6) and there have been an increasing number of complex projects like the one with which I myself have been associated for some years now. Our team includes a biochemist, a neurologist versed in electroencephalography, psychoanalysts, a psychologist and a mathematician. Although we represent such diverse disciplines, we have felt rewarded by the emergence of some suggestive correlations, some of them quite unexpected, in the findings we have each reported.

The 24-hour urinary steroid and the uropepsin output, for instance, of a manic-depressive woman were observed at weekly intervals over a period of 12 months (7) (Fig. 1). During the months of a manic phase, the 17-hydroxycorticoid output was extremely low although the ketosteroids were at high normal levels. In response to ACTH administration there was a sharp rise in 17-hydroxycorticoid excretion with only a slight increase in the excretion of 17-ketosteroids. The 17-hydroxycorticoid level rose spontaneously on two occasions during the manic period: once on the day the patient left the mental hospital for the first time in 5 months and again following a stormy visit home (8, 9). The biochemist, who could not give an endocrinological explanation for the unusual steroid findings, suggested that she be given a dose of 100 mgm. of hydrocortisone by mouth per day for 3 successive days. This resulted in an increase of 17-hydroxycorticoid excretion of the same order of magnitude as that observed in normal subjects, thus indicating that it was unlikely that the low to absent urinary 17-hydroxycorticoid excretion had resulted from a

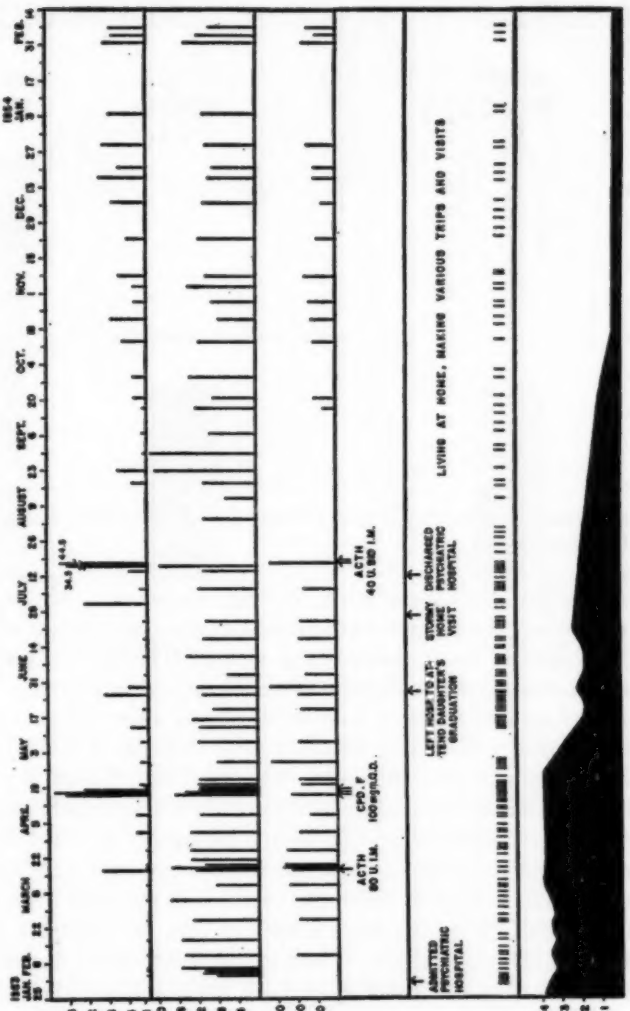

FIG. 1. 24-hour urinary steroid and uropepsin output of a cyclothymic woman.

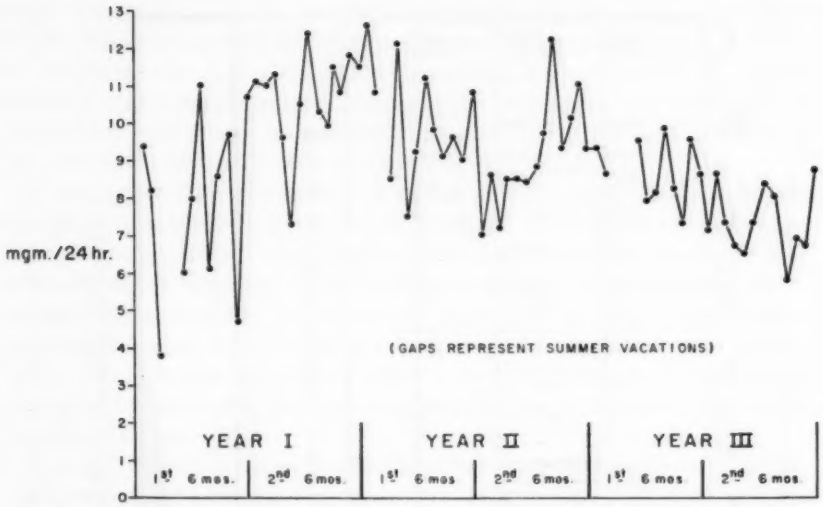

FIG. 2. Times series for bi-weekly 24-hour urinary 17-hydroxycorticoid means during three years of psychoanalysis.

disturbance in intermediary metabolism. During the period of the patient's psychological recovery the 17-hydroxycorticoid excretion rose to normal levels while the 17-ketosteroid levels showed little change. The 24-hour uropepsin figures showed an average value slightly above the upper normal range during her period of over-activity but were considerably lower after her recovery.

A male patient with a history of duodenal ulcer including a severe gastrointestinal hemorrhage was observed before, during and after 3 years of psychoanalysis (10, 11). Volume, creatinine, urinary 17-hydroxycorticoid and uropepsin determinations were carried out by the laboratory on daily 24-hour collections of urine (divided into 12-hour specimens representing the day and the night collections). A total of over 1100 twelve-hour specimens was examined. The mathematician on our team suggested that we construct a time series for bi-weekly 24-hour urinary 17-hydroxycorticoid means during the 3 years of observation (Fig. 2). Although he knew nothing about the course of the psychoanalysis, he pointed out that the curve showed a progressive damping down of the fluctuations. The peak during the last 3 months of the second year exactly corresponded to the patient's consideration and final refusal of an academic position actually offered to him in a distant area which would have necessitated termination of his analysis.

The psychologist of the team administered a Rorschach before and after the conclusion of the psychoanalysis. In general, the perception of color indicates the subjective awareness of feelings whereas answers based primarily on outline

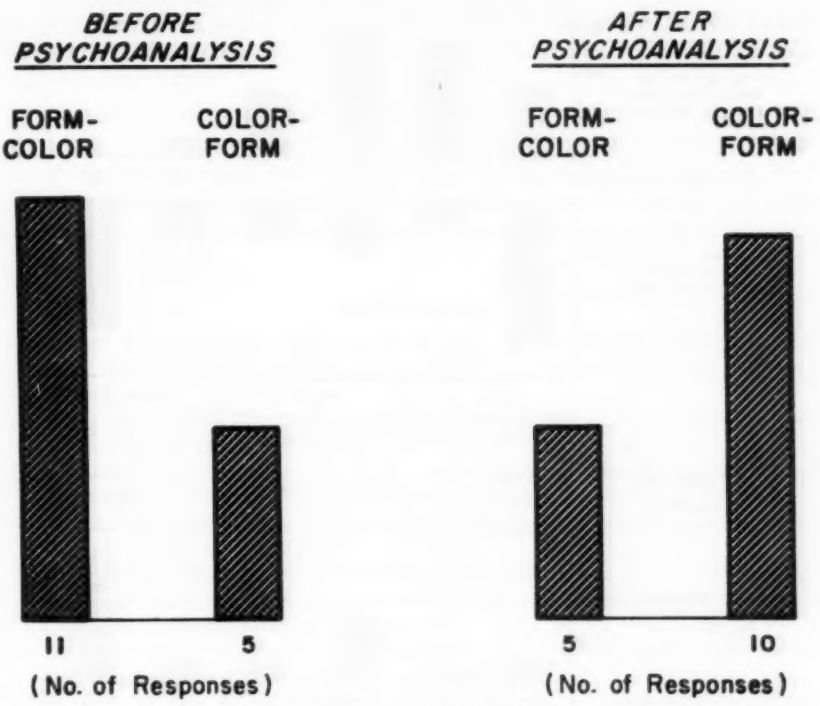

FIG. 3. Rorschach color ratios.

or form correspond to emotional control. The ratio of color to form had increased considerably by the time the analysis had been completed (Fig. 3). The psychologist interpreted the Rorschach as indicating that the patient had become more assertive, more expressive and less preoccupied with his own phantasies.

The psychoanalyst's review of the clinical material indicated that there had been an introductory phase, a middle phase, and a phase of termination in the course of the patient's relationship to his therapist. The percentage of total number of 24-hour urinary 17-hydroxycorticoid values above 12 mgm. and below 6 mgm. and of the uropepsin values above 14,000 units and below 8,000 units for each 6-month interval were determined (Fig. 4). (The values for the total period of observation were, from a statistical point of view, normally distributed, and the upper and lower limits approximately corresponded to the standard deviation for 17-hydroxycorticoids and for uropepsin.) During the introductory phase of the analysis (first 6 months) the 17-hydroxycorticoid levels fluctuated rapidly from unusually high to relatively low levels. After about 6 months, the low values dropped out and the high values were sustained

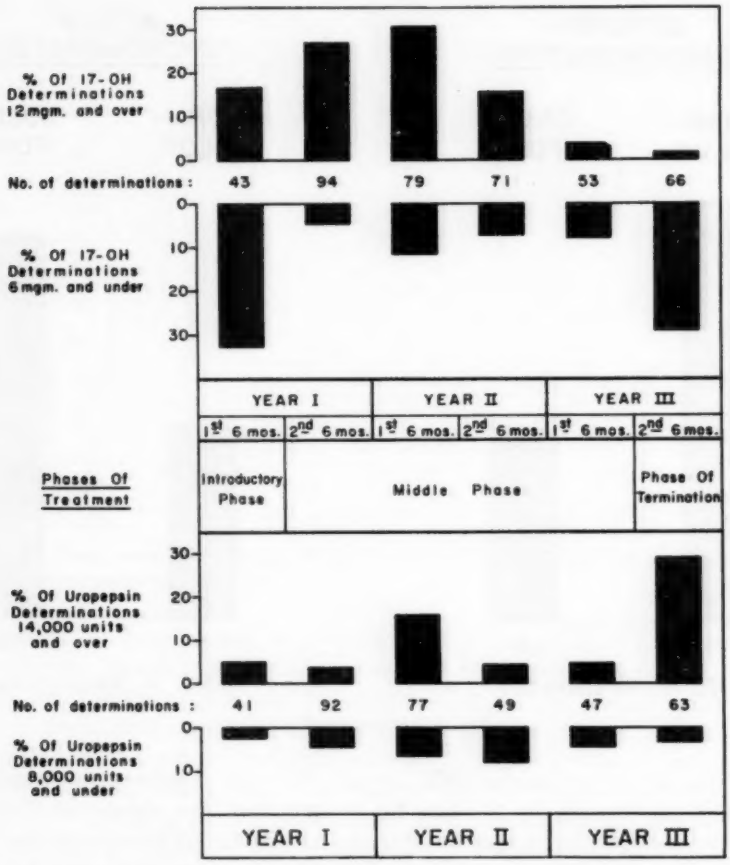

FIG. 4. Percentage of high and of relatively low 17-hydroxycorticoid and uropepsin values, with phases of the treatment during three years of psychoanalysis.

at levels usually associated with Cushing's syndrome, although this patient had no clinical evidence of endocrine abnormality. The subsequent months of the analysis were accompanied by a progressive dropping out of unusually high values and a damping down of the fluctuations. During the phase of termination (the final 6 months) there was a sharp increase in the proportion of low 17-hydroxycorticoid values comparable to the situation during the first 6 months of the analysis except for the absence now of the high individual values which had characterized the introductory phase.

The uropepsin remained at what is usually considered a level characteristic of patients with duodenal ulcer. A sharp rise above his already high average

occurred during the final 3 months of the analysis in the setting of anticipated termination, but the average fell to his usual elevated level after the analysis was over.

In general it looks as though this type of research might eventually provide at least an indirect type of validation for psychophysiological hypotheses which deal with complex data, with multi-variables, and cannot be tested by fully controlled experimental manipulation. Whether or not we are on the right track will no doubt be quite clearly determined during the course of the next 25 years.

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THE EFFECT OF INTESTINAL MOTILITY ON THE FORMATION OF ADHESIONS[1]

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The problem of peritoneal adhesions, with regard both to cause and prevention, has often been studied in the past. Work on prevention by means of: steroids (1), fibrinolysis (2), grafting (3), instillation of air (4), clot prevention (5), instillation of hypotonic solution (6, 7), and prevention of infection (8) has produced results of varying success. The early literature, to 1942, has been reviewed formally by Boys (9).

Prevention by increasing intestinal motility was discussed as long ago as 1902, 1908 and 1914 (10–14). In 1949, Schiff and Goldberg (15) demonstrated that the increased intestinal motility produced by prostigmin resulted in a significant decrease of both the number and density of adhesions, as compared with the effect of decreased motility due to atropine. The experiments reported below are quite similar to those of Schiff and Goldberg but the experimental method has been revised.

DISCUSSION OF METHOD

The test of the hypothesis that increased motion of the intestines decreases the tendency for adhesions to form or to persist is not new. However, a crucial part of the experiment, the creation of a situation conducive to the formation of adhesions, has been changed. A classic method of controlled production of adhesions involved the instillation of sterile talc. This was the method used by Schiff and Goldberg (15), as well as by many others. However, the spread of this powder within the peritoneal cavity must be hard to control and must vary widely from dog to dog. In addition, non-absorbable glove powder is now seldom used in the operating room and thus represents a truly artificial type of adhesion and one caused by a foreign body. Therefore, a new method was sought.

Since adhesions in humans seem to result from the handling of peritoneal-covered structures by the surgeon without evidence of introduction of foreign substances, trauma seemed to be the method of choice. This can be quantitated and controlled better than other methods, and from this standpoint the method of serosal stripping (1, 16, 17) represents an improvement. Although the area involved in removal of the serosa from gut surface can be accurately measured,

[1] Supported by a United States Public Health Service Institutional Cancer Teaching Grant.

this degree of trauma is severe compared to that resulting from ordinary surgical handling.

PROCEDURE

A total of 51 adult mongrel dogs was used in this study. After premedication with morphine and atropine the animals were anesthetized with ether and an endotracheal tube was inserted. Anesthesia was maintained by use of a mechanical respirator. Incision for the adhesion-producing operation was the right rectus, from the umbilicus to about 10 cm. below it. Reoperations were done with midline or left rectus incisions. Operating time was about 30 minutes.

The method of brush trauma was pioneered by Dr. J. R. Cantrell in 1957 (7). This consists of stroking a given portion of gut a given number of times with a sterile surgical scrub brush. Thus, by regulating the number of strokes, the degree of trauma can be changed. This method was used on sixteen dogs in order to test its effectiveness. It was found, however, that the brush-produced trauma was so severe that extensive and dense adhesions would form in all dogs despite other conditions of the experiment. This preliminary study showed that motility might have a tendency to lighten the adhesions, but the comparison was relatively unconvincing.

A new method of producing less intense but measurable trauma was therefore developed. This consisted of marking the distal end of the terminal ileum 20 and 60 cm. from the cecum with a black silk suture, thus identifying a 40 cm. segment of gut at 20 cm. from the end of the small intestine. This segment was then stroked four times on the anti-mesenteric surfaces with a sterile surgical sponge moistened with physiologic saline and then replaced into the peritoneal cavity. The first and second strokes would dry the serosa of peritoneal fluid, while punctate hemorrhages would always be present after the fourth.

After ten to fourteen days the abdomen was re-explored. In some animals a second procedure was performed; others were sacrificed.

The ileum was traumatized as described above in a series of 31 dogs, divided into three groups. Ten of these dogs were given no drug following operation, while eleven operations were followed by a course of prostigmin, 1 mg. every eight hours for three days, injected subcutaneously, the first dose being given immediately after operation. The remaining ten dogs were given atropine sulfate, 1 mg. every eight hours for three days, injected subcutaneously.

All of the control dogs were reoperated upon at two weeks following the trauma. Of the group given prostigmin, however, four dogs died within ten days of operation and were not included in the series. All four deaths were thought to be due to prostigmin poisoning. The remaining eleven dogs were reoperated upon or were sacrificed at about two weeks following the trauma. Nine of the ten dogs which had been given atropine lived to be sacrificed or

reoperated upon, while one died but survived long enough to be included in the series. Thus, the results are based on ten controls, eleven dogs receiving prostigmin, and ten dogs receiving atropine.

Each of the dogs receiving prostigmin had several liquid stools during the second and third days of treatment, while the dogs receiving atropine had scanty hard stools. In addition, many of the dogs receiving atropine became vicious after about 7 mg. of the drug and treatment was discontinued following this evidence of atropine intoxication in the interest of safety of both experimenter and subjects.

RESULTS

The interpretation of the results is based upon a grading system artibrarily quantitating two characteristics of the adhesions. Rating systems were devised to describe both the amount of traumatized segment involved and the density of the adhesions.

Density: These ratings describe the adhesions seen;

0. No adhesions seen.
I. *Filmy*; no deliberate effort is required to pull the adhesions apart.
II. *Medium*; the adhesions can be pulled apart without stripping serosa.
III. *Heavy*; the adhesions can be cut apart without danger of perforation of the gut.
IV. *Extreme*; the loops of intestine involved have lost their identity, and thus separation carries the peril of perforation of one or both lumens.

Extent: Ratings are based on the proportion of the traumatized surface involved in the adhesions:

0. None to one-tenth of the length of the segment of gut is involved.
1. Less than one-third of the segment is involved.
2. Less than two-thirds of the segment is involved.
3. More than two-thirds of the segment is involved.
4. Not only is approximately 100 per cent of the segment involved, but there is doubling and redoublng of the segment and adherence of the gut into a mass.

A plus (+) is added to the above when the numbers alone fail to describe the extent. Thus, a plus denotes rotation of a segment into the mesentery, extensive omental involvement, or involvement of non-traumatized gut, the latter being a rare occurrence and usually consisting of filmy, violin-string adhesions.

The results of each operation as measured by the above system were then summed up, the plus counting one-half. Thus, a rating of II, 3 = 5 and II, 3+ = 5½. In this way, a figure was obtained by which an average grade of

adhesion could be described for each experiment. The figure, however, has no meaning except that of a number selected to replace words as a means by which results can be better compared.

The results of the three groups given sponge trauma are presented using the above grading system. Photographs, although made, were not relied upon, since a photograph shows only one view of a three-dimensional mass and gives no indication of the density of the adhesion. It is felt that a brief description of

TABLE I

Results in Control Group

Dog	Time Interval (Days)	Density	Extent	Sum
ES 117	14	II	2+	4½
ES 118	14	0	0+	½
ES 119	13	0	0+	½
ES 120	14	II	1+	3½
ES 121	13	II	2+	4½
ES 151	15	II	1+	3½
ES 152	15	I	1+	2½
ES 153	14	II	1+	3½
ES 154	14	I	1	2
ES 155	13	0	0	0
Group average sum				2.5

The *Time Interval* refers to the postoperative day on which the dog was explored for adhesions. See text for explanation of *Density*, *Extent*, and *Sum*, as well as the summary of effects.

TABLE II

Results with Prostigmin and Hypermotility

Dog	No. of Mg. Given	Time Interval (Days)	Density	Extent	Sum
ES 122	9	15	0	0+	½
ES 124	9	15	II	2	4
ES 125	9	15	0	0+	½
ES 128	9	14	I	2	3
ES 138	9	12	0	0+	½
ES 161	9	16	0	0+	½
ES 162	9	16	I	1	2
ES 163	9	16	0	0	0
ES 164	9	16	0	0+	½
ES 165	9	11*	0	0+	½
ES 168	9	13	0	0	0
Group average sum					1.09+

* Post mortem.

The *Time Interval* refers to the postoperative day on which the dog was explored for adhesions. See text for explanation of *Density*, *Extent*, and *Sum*, as well as the summary of effects.

TABLE III

Results with Atropine and Hypomotility

Dog	No. of Mg. Given	Time Interval (Days)	Density	Extent	Sum
ES 130	7	13	II	1	3
ES 131	8	10*	II	4+	6½
ES 132	6	13	II	4+	6½
ES 133	7	13	II	1+	3½
ES 134	9	13	II	3+	5½
ES 135	9	13	II	4+	6½
ES 136	8	13	II	4+	6½
ES 137	9	13	II	3+	5½
ES 166	9	15	I	1+	2½
ES 167	8	13	IV	3+	7½
Group average sum.....					5.35

* Post mortem.

The *Time Interval* refers to the postoperative day on which the dog was explored for adhesions. See text for explanation of *Density*, *Extent*, and *Sum*, as well as the summary of effects.

each experiment, as presented in Tables I, II and III, gives a more concise and exact view of the total situation.

SUMMARY AND CONCLUSIONS

With the use of a method of producing measured, similar trauma to the peritoneal surface of the small intestine in dogs, adhesions were formed after fourteen days in seven of ten so treated (Table I). Filmy to medium strength adhesions involving one-third to two-thirds of the segment prevailed in the control group.

When, in addition, intestinal contraction was stimulated by the administration of prostigmin, adhesions were observed in only three of the eleven so treated (Table II). Only one of these had adhesions of medium density and the segment in these three dogs was from one-third to two-thirds involved.

When, however, atropine was administered in addition to the measured trauma, adhesions were observed in all ten animals so treated (Table III). All but one of these dogs had adhesions of at least medium density and seven of them had involvement of more than two-thirds of the segment. By inference, then, the use of drugs which inhibit peristaltic activity is undesirable following laparotomy, and paralytic ileus due to any cause may be presumed to enhance the persistence of intraperitoneal adhesions.

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VARIABILITY OF CHOLESTEROL LEVELS IN INDIVIDUAL JOHNS HOPKINS MEDICAL STUDENTS, WITH OBSERVATIONS ON STOPPING SMOKING, VITAMIN B_{12} ADMINISTRATION AND ACUTE INFECTION[1]

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In previous studies concerning the cholesterol levels of the Johns Hopkins medical students, using the Buell modification of the Bloor method, we have found a wide range of normal values: from 108 through 407 mg. per 100 cc. at first test among 612 men and women (1, 2). Although the mean cholesterol level tended to rise with age for the group, the factor of age alone did not account for the wide differences in cholesterol level, nor did age always parallel height of cholesterol in a given subject on repeated tests. Hypercholesteremic individuals[2] were found in all groups studied over the age of 20 years. These high levels did not seem attributable to diet, but showed a high degree of association with a history of coronary heart disease in the fathers of the subjects. Moreover, we found that those with higher cholesterol levels were more often smokers than were those with lower levels (3). Over a period of time, there was a marked variation in level in some subjects, while in others, cholesterol remained quite stable. In an occasional person, the appearance of hypercholesteremia seemed to be associated with stress, and elevation of cholesterol level with stress has been observed by others and by us (4–9). All of these findings taken together suggest that there is much more to be learned about the biological variables affecting the cholesterol level in a given individual. The present report concerns intensive longitudinal study of the effect of three such variables on the cholesterol level of a few individuals in a particular environment, rather than the more usual cross-sectional study of a large population.

I. PRELIMINARY OBSERVATIONS OF THE EFFECTS OF STOPPING SMOKING ON CHOLESTEROL LEVEL

Statistical analysis of the relationship of smoking habits to cholesterol levels in Johns Hopkins medical students showed a positive association between the habit of smoking and the height of the cholesterol level (3). In that study, more smokers than expected had cholesterol levels of 250 mg. per 100 cc. or

[1] This study was supported in part by Research Grant H-1891, National Heart Institute, and in part by the Tobacco Industry Research Committee.

[2] Those with cholesterol of 300 mg. per 100 cc. or more.

above while more nonsmokers than expected had levels below that value, when the highest of from one to five cholesterol determinations for each subject was used. The ages of the four groups were very similar, and we have since confirmed the fact that there was no significant difference in the age distribution of the smokers versus the nonsmokers at each cholesterol level or in the age distribution of the low versus high cholesterol groups divided into smokers and nonsmokers. Other population surveys are necessary to corroborate the existence of such an association and to determine its extent. If these findings are substantiated, it remains to be determined whether smoking over a period of time raises the serum cholesterol level, or whether individuals with higher cholesterol levels are more likely to be regular smokers.

A way to throw more light on the entire question of the relationship of smoking to cholesterol level would be to alter the smoking habits of a group of subjects over a long period of time and observe the effect on cholesterol levels, if any, compared with a control group. One approach would be to study the cholesterol levels of boys before they start to smoke and to follow them over the years, as they became smokers or remained nonsmokers. However, in order to decide the question, it would be necessary to establish a randomly selected control group of "potential regular smokers" who refrain from smoking for the purposes of the study only. Admittedly, the execution of this plan presents many difficulties. Another approach would be to investigate the effect of stopping smoking on the cholesterol levels of regular smokers, to find out whether or not these levels tend to fall after smoking has stopped. Ideally, subjects with both high and low cholesterol levels should be studied, for they might respond differently to stopping smoking. In September, 1957, it was decided to attempt such a pilot experiment concerning the effect of stopping smoking on the cholesterol levels of regular smokers.

A. Method

A notice was posted, stating the purpose of the study as given above and asking for volunteers to stop smoking from the classes of 1958, 1959, and 1960. The qualifications and experimental plan were outlined as follows:

"1. All subjects are to be male students who have smoked cigarettes daily for a period of at least one year, and preferably three.

2. Blood for cholesterol levels is to be drawn twice weekly for a four-week control period.

3. Smoking is then to be completely stopped. A transition period of a week will be allowed for stopping.

4. Weekly cholesterol levels are to be obtained during the next six weeks after which levels will be taken less frequently during the rest of this academic year.

5. A control group of regular male smokers will be studied in the same way in order to control the effects of season and academic life on cholesterol level. These students will not be asked to change their smoking habits; in fact, they will be expected to continue smoking as usual throughout the academic year.

6. Subjects will be paid $1.00 per vena puncture."

Since no candidates were immediately forthcoming, the second year class (which has more free time than the upper classes) was further approached by a brief announcement. As a result, seven persons from the class of 1960 volunteered to stop smoking and five were accepted; the other two did not meet the qualifications.[3] One of the five students accepted was a woman; although an exception, she was included because of the small number of volunteers.

The volunteers to stop smoking registered between September 25 and October 3, 1957.[4] The control subjects were not registered until somewhat later (one volunteered on October 8 and four others on October 31, 1957[5]). All filled out a detailed questionnaire concerning smoking habits, including age begun, periods of nonsmoking, total period of smoking, specific kinds of smoking, amount of each kind smoked over the years, and whether or not the subject inhaled. An appraisal of the fat content of the diet was also made.

Serum cholesterol levels were determined in the Clinical Chemistry Laboratory of the Johns Hopkins Hospital by the Bloor method as modified by Sackett and by Buell. This method has been described in detail and its reliability in comparison with other methods established (2). In the present study, duplicate determinations were made throughout. As previously described, the two blood samples were sent to the Clinical Chemistry Laboratory under different names to prevent identification of the pairs of tests.

B. Time table and definition of terms

The studies comprising the five parts of this paper were closely interrelated. They were continuous in point of time, uniform as to method and were made on the same subjects in varying groups. All observations were made between September 25, 1957 and June 5, 1958. For the studies in Part I, the subjects were divided into the "SS group," (those who volunteered to stop smoking) and the "C group," (the control subjects who continued smoking as usual). Successive time periods were defined as follows:

Period I. Control period. Both SS and C groups smoked the customary amount. Duplicate cholesterol determinations, blood pressure, heart rate and body weight were obtained biweekly on eight occasions over a total period of 23 to 27 days (Subject 10, 36 days).

Period II. "Stop smoking" experiment. Five SS subjects (1–5) either gave up smoking entirely or attempted to do so. Five C subjects (6–10) smoked as usual. Period II ended before the Christmas vacation.

Period III. Control period beginning after the Christmas vacation. Subjects 1, 4, and 5 no longer under observation. Subjects 2 and 3 now smoking, as were all C subjects.

Periods II and III combined. These periods were combined for the C subjects 6–10 to establish a long control period with adequate sample size prior to the administration of Vitamin B_{12}. (For SS Subject 3, Periods I and III were similarly combined.)

Period IV. Vitamin B_{12} administration. Although this period pertained entirely to the studies under Part II, it will be described here for the sake of continuity.

A. Transition period: the two pairs of cholesterol determinations obtained within 14

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[3] One was a man who had already stopped smoking for three months just prior to the experiment and the other was a woman with small, fragile veins who was not sure she could stop smoking because her husband was a regular smoker.

[4] An additional man from the class of 1961 (Subject 61107) who wanted to stop smoking was registered in January, 1958.

[5] These four were solicited by our student assistant from a list of regular smokers because of the need for more control subjects. No matching was attempted and no special selection was made.

days (Subject 6, 18 days) after vitamin B_{12} administration was begun were not pooled with the later values. Individual subjects started taking B_{12} on either Feb. 6, 7 or 11.

B. Later period: includes all other cholesterol values while taking vitamin B_{12} until the end of the school year.

Subject 11 entered the study while the original subjects were in Period III.

His time table was as follows:

Period I Control period: smoked as usual Jan. 28–Feb. 21.

Period II "Stop smoking" experiment: Mar. 4–May 1.

Period III Control period: smoked as usual May 8–May 28.

The exact overall time period of observation for each subject from the first to the last cholesterol determination inclusive is given in Table XI.

C. Results of "stop smoking" experiment

1. ***Composition of experimental and control groups.*** There were 64 men and 7 women in the class of 1960 as it entered the second academic year in the fall of 1957. As determined in a survey of smoking habits made during the spring of 1957, 28 men and four women, or 32 of the 71 students, were regular smokers. The four men and one woman who volunteered for the "stop smoking" experiment from this class and the five control subjects, who were all men, were self-selected from this group of regular smokers.

The characteristics of the experimental (SS) and the control (C) subjects are presented in Table I. The two groups were similar in age, in number of cigarettes smoked per day, and in dietary habits. On the average, the control group had smoked somewhat longer, although there was considerable individual variation within both groups. However, when the four men in the SS group are compared with the C group as to nutritional status and body build, the SS group, although averaging almost an inch taller, was 12.6 lbs. lighter.[6] The cholesterol levels were quite different in the two groups. The means of levels obtained on three occasions[7] over the course of a year indicate that all those who volunteered to stop smoking had relatively low cholesterol levels to begin with, while those who agreed to act as controls had a wider range of cholesterol levels including several subjects with quite high values.[8] Actually, the SS group differed not only from the C group but from the entire class in regard to distribution of cholesterol values (Table II).

Circulatory measurements for the SS and C groups also differed as to mean systolic pressure, but resembled each other as to diastolic pressure and heart

[6] Subject 11, the additional SS subject, resembled the C group in body weight and ponderal index.

[7] Two occasions, Subject 3.

[8] The two volunteers for the stop smoking experiment who were rejected because they did not meet qualifications (see above) also had relatively low cholesterol levels. The man and woman each had six determinations on four occasions between October, 1956 and October, 1957, with ranges of 185–217 and 185–239 mg. per 100 cc. respectively.

TABLE I

Characteristics of Subjects

	SS Group						C Group						Additional SS Subject
	Those who volunteered to stop smoking						Controls who did not stop smoking						
Study No.	60227	60133	60158	60166	60123	Mean of 4 Men	60134	60107	60118	60144	60171	Mean of 5 Men	61107
Subject No.	1	2	3	4	5		6	7	8	9	10		11
Sex	F	M	M	M	M		M	M	M	M	M		M
Marital Status	S	S	S	S	M		S	S	M	M	M		S
Age at Start of Experiment	21–6	22–0	24–2	23–3	22–11	23–1	23–3	23–11	23–0	22–1	22–11	23–0	22–2
Smoking history													
Age begun	16–17	17	21	16	17	18	17	22½	15	16	12	16½	18
Total years	4	5	3	7¼	3	4½	5¾	1½	8	6	9¾	6¼	4¼
Cigarettes/day	12	20	10	40	20	22½	20	15–20	20	20[1]	20	20	11–19
Years of that amount	4	4	2	3	1	2½	4	1	4	4	8	4	4
Dietary history													
Butter pats/day	1	1+	1–2	1	3	1–2	2–3	2–3	1–2	0	1	1–2	1
Glasses whole milk/day	½	1–2	3	3	2	2+	½	2–3	2	2–3	0	2–	3
Cuts down on fat	Yes a	Yes b	Yes c	? d	Yes b, c, e, f	Yes	Yes b, c	Yes g	Yes b, c	Yes a	Yes g, h	Yes	Yes b
Nutritional status													
Height/inches	62¼	73¼	70¼	71½	73¼	72.0	72	71	69	71	73	71¼	72⅛
Weight/lbs.[2]	120	166	150	170	165	162.8	188	175	165	186	163	175.4	179
Overweight/lbs.[3]	0	−2	−6	+6	−4	−1.5	+24	+15	+14	+28	−6	+15	+16
Ponderal Index[4]	12.60	13.30	13.20	12.90	13.30	13.18	12.50	12.70	12.60	12.45	13.35	12.72	12.75

Ponderal Index[4]	12.60	13.30	13.20	12.90	13.30	13.18	12.50	12.70	12.60	12.45	13.35	12.72	12.75
Cholesterol levels													
Fall, 1956[5]	190	185	—	177	190	184.0	273	243	273	258	210	251.4	—
Spring, 1957[6]	250.5	190.0	222.0	187.5	173.5	193.2	303.0	181.0	229.5	273.0	213.5	240.0	—
Fall, 1957[6]	196.5	186.0	193.0	219.0	202.5	200.1	300.0	248.0	286.5	278.0	205.0	263.5	160
Mean	212.3	187.0	207.5	194.5	188.7	194.4	292.0	224.0	263.0	269.7	209.5	251.6	—
Circulatory measurements													
Systolic Pressure[2]	122	137	125	128	130	130.0	127	127	122	119	120	123.0	123
Diastolic Pressure[2]	73	81	61	86	75	75.8	78	78	77	69	70	74.4	71
Heart Rate[2]	114	73	78	81	78	77.5	83	72	85	84	67	78.2	86

[1] Subject changed from 20 cigarettes to 6–8 pipes a day and an occasional cigarette just before entering the experiment.

[2] Mean of eight tests.

[3] Based on tables published by the Association of Life Insurance Directors and Actuarial Society of America, New York, 1912, p. 38.

[4] Ponderal index = height/$\sqrt[3]{\text{weight}}$. See Table VIII, Appendix 2, "Atlas of Men" by William H. Sheldon.

[5] Single sample.

[6] Mean of duplicate samples.

a. To avoid gaining weight.
b. Cuts away fat from meat.
c. Dislikes fatty foods and/or fat on meat.
d. Eats very little ice cream.
e. Avoids fried foods and fats because of acne.
f. Uses vegetable oils in cooking.
g. No cream.
h. Drinks only skim milk.

TABLE II

Distribution of Mean Cholesterol Levels for 64 Men in the Class of 1960[1]

Cholesterol Level Mg. %	All Men		SS Men	C Men
	N	%	N	N
175–199	13	20.3	3	0
200–224	21	32.8	1	2
225–249	19	29.7	0	0
250–274	9	14.1	0	2
275–299	2	3.1	0	1
All levels	64	100.0	4	5

[1] The SS and C groups of men are included in the total of 64 men.

The single reading in the fall of 1956 and the mean of duplicate samples in the spring of 1957 have been averaged for 62 men. Two men had no single reading in the fall of 1956, so that the mean of the duplicate samples in the spring of 1957 was used as the best estimate of their mean.

rate (Table I). It is clear that the two groups were somewhat different, and while well-matched as to age, amount smoked per day and dietary habits, were not entirely comparable constitutionally, particularly in regard to cholesterol levels. On the basis of these variables, Subject 10 might be considered as a comparable control for Subjects 2, 3, and 5, while Subject 7 is a fairly good control for Subject 4. Subjects 1, 6, 8, and 9 are essentially unmatched.

2. *Success as to stopping smoking and subjective effects.* The subjects in the experimental group showed varying degrees of success in stopping smoking, despite their desire to do so. Only one subject stopped smoking completely for the entire eight weeks.

Experience in this regard was as follows:

Subject 1 stopped completely for more than three weeks, then smoked several cigarettes one evening and continued to smoke about one a day until she withdrew from the experiment after five weeks of Period II.

Subject 2 smoked 1–2 cigarettes on three occasions the first week, then stopped completely for the next seven weeks save for 6–8 cigarettes during Thanksgiving vacation.

Subject 3 stopped smoking completely for eight weeks.

Subject 4 smoked about 10 cigarettes a day for the first week, then stopped completely except on weekends, when he smoked a reduced amount. He withdrew from the experiment after seven weeks of Period II.

Subject 5 stopped completely for five weeks except for 2 cigarettes over Thanksgiving vacation, returned to smoking the weekend of the sixth week and withdrew from the experiment.

Subject 11, after tapering off the first week, stopped completely for six weeks except for two cigarettes one weekend. He smoked at the end of the seventh week of Period II, smoked a variable amount for the next 2–3 weeks, and had resumed regular smoking by the tenth week.

Subjects 2 and 3, given their choice, elected to return to smoking during the Christmas vacation after 7–8 weeks of not smoking.

The subjective effects of stopping smoking deserve brief mention. Subject 3 described his reactions as follows: "For about one week after stopping I encountered 'physiological difficulties' in stopping. I felt sluggish and tired. I felt that I needed a cigarette to 'pick me up.' I wanted a cigarette 'all day long.' Later on, I continued to have difficulties in stopping but I would classify these as psychological. I wanted to smoke when under 'nervous stress' —when studying for an examination, when taking an examination. I also wanted a cigarette when relaxing after an exhausting ordeal—e.g. after a difficult examination. I wanted to smoke when in conversation, particularly at a party. I felt very clumsy and awkward socially without a cigarette in my hand. The urge to smoke was most severe when I was depressed. When relaxed, contented, living a regular routine, and more or less by myself I felt no desire to smoke." Two subjects spontaneously reported sleep changes in the first week:[9] Subject 2 felt intensely sleepy two days after stopping smoking, and actually slept 14–16 hours at one stretch. Subject 11 noted that he was sleepier after supper and in the morning than when smoking. Other early reactions included the constant desire for a cigarette, compulsive eating of cough drops or Life Savers, the use of chewing gum, and more than normal hunger. As Subject 11 said, "The first two weeks were hard, then it was not so hard," but after the fifth week it became increasingly difficult again, "particularly in this environment." Only Subject 5 stated at the end of three weeks that he had had no change in appetite or trouble stopping smoking. In fact, he had been less nervous than he was while smoking. Nevertheless, within three weeks of that statement he had returned to smoking. It is of interest that five of the six SS subjects gave up the battle after rather similar periods of time: five to seven weeks, and Subject 3 did not resist much longer.

3. *Effect on mean cholesterol level, total variability and laboratory reproducibility of cholesterol.* The effects of completely or partially stopping smoking on cholesterol levels in this experiment are shown in Table III and Figure 1. It will first be seen that there were no large changes in mean level in either direction. In particular, only one of the five original SS subjects (Subject 4) showed a decrease in mean cholesterol level and that decrease was not significant. The other four subjects each showed a modest rise in mean cholesterol level in Period II as compared with Period I which was only significant in Subject 2 ($P = .05$). This rise approached significance in Subject 3, who also showed a significant fall in mean cholesterol after smoking was resumed ($.001 < P < .01$ when Period III is compared with Period II). Any importance attached to these findings is nullified by the fact that all five of the C subjects also showed a rise in mean cholesterol level between Period I and II (Figs. 2–4). This rise approached significance in Subject 6 and one individual (Subject 8) showed a significant rise-fall effect over the three periods like that of Subject 3 ($.02 < P < .05$ for Period I versus Period III and Period II versus Period III). It is likely that time of year or seasonal pressures had more to do with this trend than any effect of stopping smoking (9). The additional SS individual (Subject 11) also showed no significant change in mean cholesterol level from stopping smoking.

[9] Subjects were asked something like "How are things going?" but leading questions were avoided.

TABLE III

Summary of Cholesterol Data in "Stop Smoking" Experiment

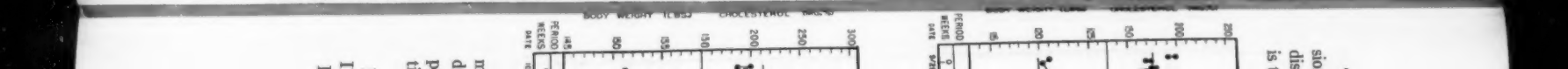
	Subject No.	Length of Time in Days	Cholesterol Levels in mg. per 100 cc. Total N	Mean ± S.E. Mean	s_T	s_e	Length of Time in Days	Total N	Mean ± S.E. Mean	s_T	s_e	Length of Time in Days	Total N	Mean ± S.E. Mean	s_T	s_e
			Period I Control Period (Sept. 25–Oct. 29)					Period II "Stop Smoking" Experiment (Oct. 24–Dec. 17)					Period III Control Period (Jan. 3–Jan. 30)			
A. "Stop smoking" group	1	23	16	176.9 ± 3.8	15.3	6.8	29	10	182.3 ± 6.7	21.2	8.0					
	2	24	16	185.1 ± 3.1	12.3	7.2	43	14	194.5 ± 3.1	11.5	2.5	1	2	186.5	—	—
	3	24	16	211.5 ± 5.4	21.8	13.7	48	16	225.8 ± 4.7	18.8	14.6†	22	8	201.0 ± 4.9	13.9	9.7†
	4	27	16	197.5 ± 5.7	22.7	17.3†	43	14	187.4 ± 2.3	8.6	4.5					
	5	27	16	183.6 ± 4.3	17.1	8.4	36	12	186.8 ± 3.8	13.3	4.7					
			Period I Control Period (Oct. 8–Dec. 5)					Period II Smoked as Usual (Nov. 7–Dec. 19)					Period III Control Period (Jan. 7–Feb. 11)			
B. Comparable control subjects	7	27	16	227.9 ± 4.3	17.1	5.7	21	8	233.8 ± 4.8	13.6	13.2†	35	12	230.6 ± 4.4	15.3	5.5
	10	36	16	190.5 ± 2.8	11.3	6.8	1	2	200.0	—	—	31	8	183.8 ± 3.0	8.5	6.7†
C. Other control subjects	6	24	16	297.3 ± 5.5	21.9	9.5	36	10	315.5 ± 6.3	19.8	19.3†	29	6	315.8 ± 12.6	31.0	9.0
	8	27	16	265.0 ± 4.7	19.0	15.6†	21	8	283.0 ± 12.8	36.1	8.8	31	10	244.0 ± 6.2	19.6	12.2†
	9	27	16	268.8 ± 3.8	15.4	9.2	21	8	269.8 ± 6.1	17.3	13.2†	32	10	278.2 ± 6.5	20.4	8.4
			Period I Control Period (Jan. 28–Feb. 21)					Period II "Stop Smoking" Experiment (Mar. 4–May 1)					Period III Control Period (May 8–May 28)			
D. Additional "stop smoking" individual	11	25	16	209.8 ± 5.1	20.4	7.4	59	18	211.3 ± 4.7	19.8	11.6	21	8	214.9 ± 5.6	15.8	2.5
		22	14[1]	215.1 ± 4.1	15.3	7.9										

Total N: The mean and s_T are calculated on the basis of Total N, or all the observations, i.e., the sum of the unaveraged duplicate determinations. The number of duplicate tests for the calculation of s_e, the technical error of measurement, is half of the total N.

Dates: The dates for Periods I and II for Subjects 1–10 are not mutually exclusive because individual subjects entered each period at slightly different times. The "length of time in days" for each period or combination of periods is the number of days between the first pair and the last pair of duplicate cholesterol determinations, inclusive, in the specified period or combination of periods. By this definition, the "total time observed in days" during Periods I, II, and III is not exactly the sum of the "length of time in days" for Period I, Period II, and Period III separately.

[1] Excluding the postinfluenzal pair of duplicate determinations (see Table VIII).

† No significant biologic variation (s_b).



The question of whether or not the total variability of the cholesterol level, as an expression of the cholesterol stability of the individual SS subjects, was altered by decreasing or discontinuing smoking may be answered by examining the s_T values in Table III. The s_T is the standard deviation of all the observations about the mean for a given period and its

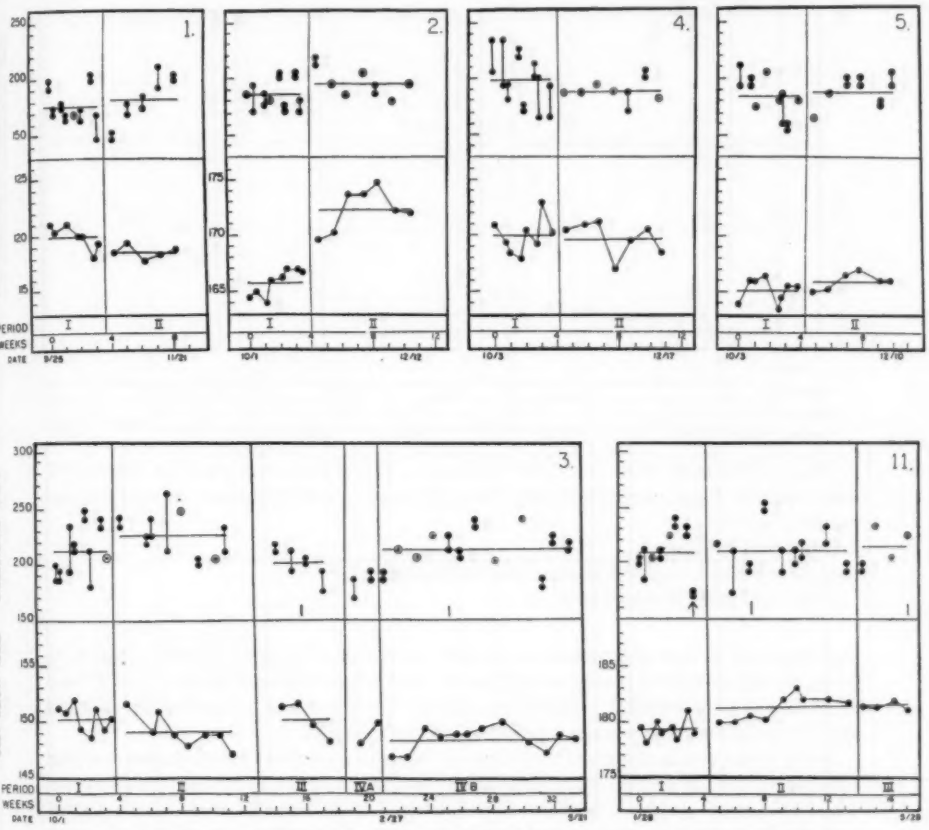


FIG. 1. The effect of stopping smoking on the cholesterol level and body weight of six medical students. The pairs of cholesterol determinations linked by vertical lines were duplicate tests from the same serum sample. The length of the vertical line is inversely proportional to the degree of technical accuracy achieved in that particular duplicate determination. A dot within a circle indicates that there was *no difference* between the duplicate tests.

I = Control period
II = Period of stopping smoking (partially or completely, see text)
III = Control period
IV = Period of vitamin B_{12} administration. The two determinations between III and IVB form the transition period IVA.
↑ = Postinfectional cholesterol determination
| = Final academic examination

cholesterol determinations, inclusive, in the specified period or combination of periods. By this definition, the "total time observed in days" during Periods I, II, and III is not exactly the sum of the "length of time in days" for Period I, Period II, and Period III separately.
‡ Excluding the postinfluenzal pair of duplicate determinations (see Table VIII).
† No significant biologic variation (6a).

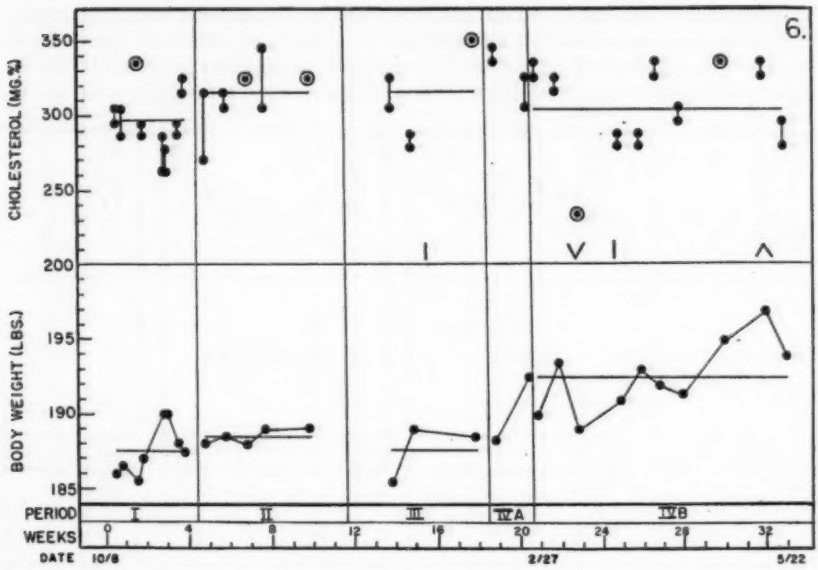


FIG. 2. Cholesterol levels and body weight in a hypercholesteremic subject (Subject 6) over a period of eight months. During Period II he was a control subject and continued to smoke as usual.

Legend as in Figure 1.

Also $\wedge$ = Cholesterol determination after mild infection

$\vee$ = Highest B_{12} blood level

size reflects all sources of technical and biologic variability. Only one individual (Subject 4) of the six who attempted to stop smoking had a significantly different (smaller) s_T for Period II compared with Period I. Among the controls, Subject 8 had a significantly different (larger) s_T for Period II when compared with Period I and III.

Study of the technical error of measurement (s_e) gave no clearcut evidence that stopping smoking affected laboratory reproducibility, although two of the six SS subjects (Subjects 2 and 4) showed significant decreases in s_e between Periods I and II. None of the control subjects showed such a decrease, and C Subject 7 showed a significant increase (s_e I versus s_e II). SS Subject 11 showed the only other significant change: his s_e for Period III was significantly lower than for either Period I or Period II.

4. *Effect on body weight, blood pressure and heart rate.* As shown in Table VII and Figure 1, Subject 2 exhibited a highly significant gain in weight after stopping smoking ($P < .001$). He experienced a mean increase of 6.6 lbs. between Period I and Period II, with a difference of 10.8 lbs. between the lowest value for Period I and the highest value for Period II. Subject 11 likewise showed a modest but significant increase in mean weight when Period II is compared with Period I ($.001 < P < .01$). Subject 1 had a small decrease in her mean weight in Period II ($P = .02$). However, she had already begun to

lose weight at the end of Period I. None of the C subjects showed any significant variation in weight between Periods I and II, nor did any of their mean circulatory measurements change significantly (Table VII and Figures 2–4). Two of the six SS subjects, on the other hand, showed a significant lower-

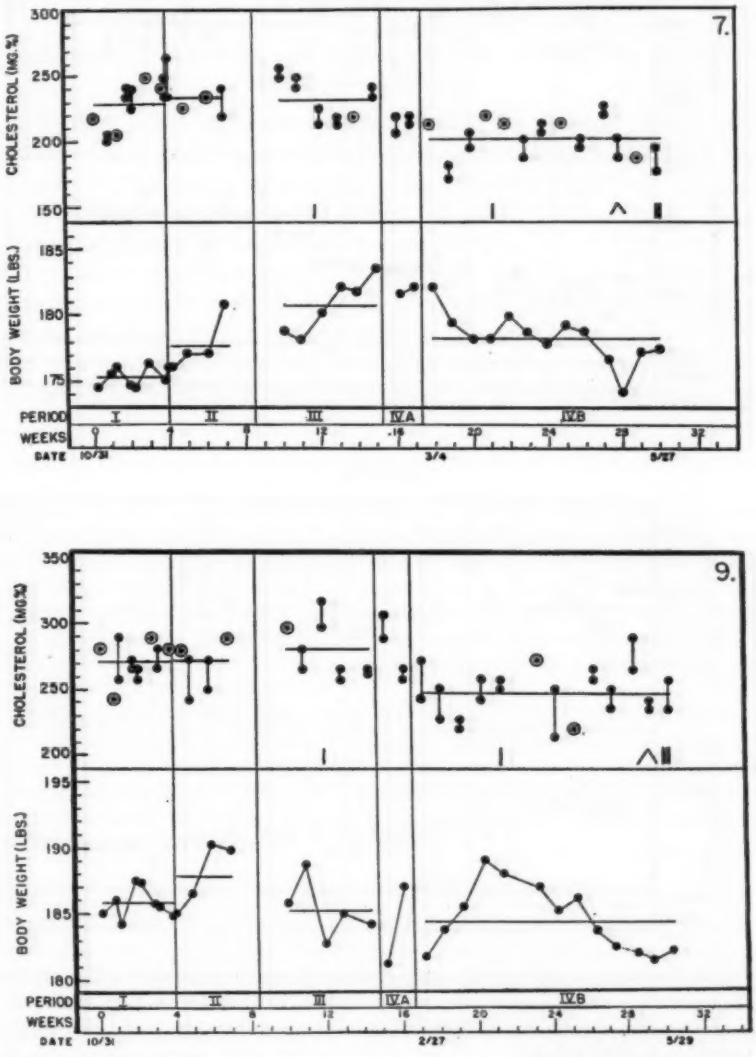
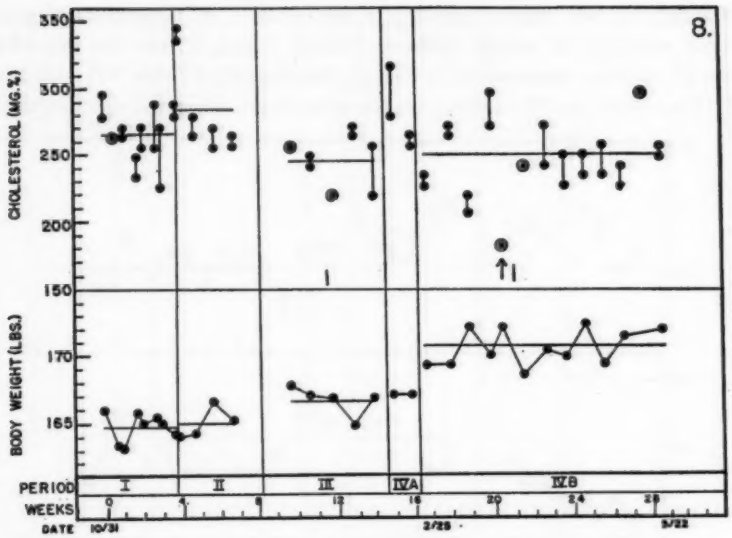


FIG. 3. Two subjects (7 and 9) who showed significant lowering of their cholesterol levels during vitamin B_{12} administration (Period IV). They also were control subjects during Period II, when they continued to smoke as usual.

Legend as in Figs. 1 & 2.



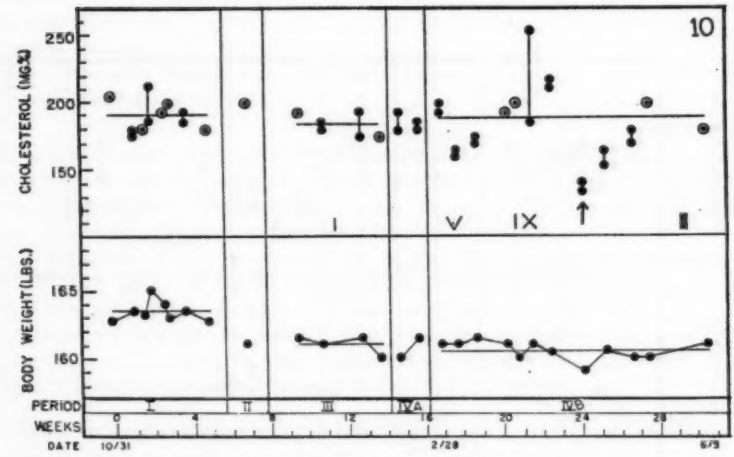


FIG. 4. Two subjects (8 and 10) each of whom showed a transitory fall in cholesterol level immediately after an acute infection, as did Subject 11 in Figure 1. Subjects 8 and 10 were also control subjects during Period II when they continued to smoke as usual.

Legend as in Figs. 1 & 2.

Also × = Disparate pair of duplicate tests.

ing of mean heart rate per minute when Period II is compared with Period I. The mean rate in Subject 1 fell from 114.3 to 101.0, while in Subject 5 the mean rate decreased from 77.8 to 71.2. There was only one other significant circulatory change among the SS subjects: the mean diastolic pressure of Subject 3 rose from 61.1 to 71.2 mm. of Hg. on stopping smoking.

II. A PRELIMINARY STUDY OF THE EFFECT OF ORAL VITAMIN B_{12} ON HUMAN CHOLESTEROL LEVELS

In 1957, Hsu and Chow reported that vitamin B_{12} deficient diets induce hypercholesteremia in rats and chicks (10). Chow and his associates have also found that, on the average, vitamin B_{12} serum levels decrease:

1. with advancing age in rats and in men (11–13).
2. during pregnancy in women and in rats (14, 15).

Since it is known that cholesterol levels rise both with age and during pregnancy in human subjects, the possibility of a human linkage between lower vitamin B_{12} levels and higher cholesterol levels appears to exist (16, 17). With Dr. Chow as our consultant, it was decided to make preliminary observations of the effect of vitamin B_{12} administered orally on the cholesterol levels of six men.

A. Method

Crystalline vitamin B_{12} was administered orally, (one 1000 microgram tablet every other day[10]) to six subjects: the five original C Subjects 6–10 plus SS Subject 3, who had returned to smoking. These men were considered especially suitable for such a study because Subjects 6, 8, and 9 had exhibited hypercholesteremic levels on one or more occasions. Since all six subjects had been under close observation for more than four months, it was thought that a rough indication of the effect of vitamin B_{12} on their cholesterol levels could be obtained without setting up a new control group. Duplicate cholesterol determinations continued to be performed as described under Section I.

Vitamin B_{12} determinations on blood serum were carried out in Dr. Chow's laboratory.[11] B_{12} in serum was liberated by heating and determined microbiologically by the method of Skeggs *et al.* (18). Blood samples were sent to his laboratory on four occasions, two before and two after starting B_{12} administration. Only on the fourth test were blind duplicate determinations obtained, although on the second test the technician voluntarily carried out duplicate determinations on five of the six samples.

B. Frame of reference and reliability of method

The mean serum B_{12} concentration for 107 healthy subjects aged 20–29 years was found to be 269.8 $\mu\mu$g. per cc. in the same laboratory and by the same method as was used in the present study (13). The minimal normal level of B_{12} in serum has been considered by several

[10] We are indebted to Merck and Co. Inc. for generously providing the tablets of crystalline Vitamin B_{12} for oral use.

[11] We should like to thank Mrs. Leida Luha for her technical assistance in making these determinations.

investigators to be about 100 μμg. per cc. (13). Both sets of duplicate tests on vitamin B_{12} blood levels in our series show the same degree of agreement as has been described on a series of 60 serum specimens analyzed in duplicate (12). While rather wide variations are to be noted in our pretreatment values (Table VI 1 and Mean 2), somewhat similar variation was observed in five Johns Hopkins University students studied for six months (12).

C. Results of vitamin B_{12} experiment

1. *Effect on cholesterol level.* The effects of oral vitamin B_{12} administration on the cholesterol levels of six male subjects are set forth in Table IV. There the mean cholesterol values for Period IVB, a 12 to 14 week period on B_{12}, are compared with the mean levels during the 10 to 14 week control period immediately preceding the vitamin administration.[12] The mean cholesterol level was lower *during* B_{12} administration than *before* in five of the six subjects. In two of the five, Subjects 7 and 9, the mean levels while taking B_{12} were 31 and 29 mg. per 100 cc. respectively below the control cholesterol level; these decreases were highly significant ($P < .001$). Subject 3 showed an insignificant rise in level while on B_{12}.

A breakdown of the cholesterol values by period for Subjects 7 and 9 is given in Table V. During Period IVB both men showed a considerably greater incidence of lower values than had appeared before. At the same time, there was a marked reduction in the higher cholesterol levels previously characteristic of them. There can be no question but that a significant change in the cholesterol level of these two subjects occurred in Period IV, concomitant with vitamin B_{12} administration. Whether this change was caused thereby, or whether it was related to some other unsuspected variable remains to be determined.

2. *Effect on vitamin B_{12} blood level.* The data on vitamin B_{12} blood levels are presented in Table VI. In comparison with the minimum normal level of 100 μμg. per cc. mentioned above, Subject 8 appears to have had an unusually low vitamin B_{12} level to start with. Despite the fluctuation in control values which are particularly marked in Subject 7, it may be seen that as a group the mean B_{12} blood level rose from 194.2 to 280.9 μμg. per cc. during B_{12} administration and that individual B_{12} blood levels rose from 15 to 159 per cent. Subjects 7 and 9, the only ones with a significant decrease in cholesterol level while taking B_{12}, also attained the highest B_{12} blood levels during its administration with means of 347 and 606 μμg. per cc. respectively. The data are somewhat marred by the fact that each of these two subjects had one high single reading during the control period. However, the final B_{12} blood level attained by Subject 9 was well above this single control value. Subject 6 reached his lowest mean cholesterol value for any duplicate test on March 13, at a moment when his B_{12} blood level may have exceeded the 280 μμg. per cc. determined a week earlier (Fig. 2).

[12] Data from Period IVA, a transition period of two weeks, are not included in this table, but are included in the individual charts (Figs. 1–4).

TABLE IV

Summary of Cholesterol Data in Vitamin B_{12} Experiment

Subject	Periods included	Length of time in days	Control Period for Vitamin B_{12} Study: Cholesterol in mg. per 100 cc. Total N	Mean ± S.E. Mean	s_T	s_e	Length of time in days	Period of Vitamin B_{12} Administration (IV B): Cholesterol in mg. per 100 cc. Total N	Mean ± S.E. Mean	s_T	s_e
3	I and III	46[1]	24	208.0 ± 4.1	19.8	12.5	84	24	213.3 ± 3.4	16.8	4.3
6	II and III	92	16	315.6 ± 5.9	23.6	16.2†	85	20	303.0 ± 7.2	32.1	6.9
7	II and III	77	20	231.8[a] ± 3.2	14.4	9.4	85	26	200.8[a] ± 2.9	14.8	6.5
8	II and III	72	18	261.3 ± 7.9	33.7	10.8	87	26	243.4 ± 5.9	29.9	11.3
								24[2]	248.7 ± 5.0	24.4	11.8
9	II and III	73	18	274.4[b] ± 4.5	19.1	10.8	92	26	245.0[b] ± 3.8	19.3	13.1
10	I, II, and III	99	26	189.2 ± 2.1	10.8[c]	6.5	98	24	184.4 ± 5.3	25.9[c]	14.7 (4.3)
								22[2]	188.6 ± 4.8	22.6[c]	15.3 (4.2)

Total N and length of time in days as in Table III.

[1] Not continuous: Period II excluded because of changed smoking status.

[2] Excluding the postinfluenzal pair of duplicate determinations (see Table VIII).

Comparison between Period IV B and Control Period: [a] Subject 7: mean cholesterol $P < .001$. [b] Subject 9: mean cholesterol $P < .001$. [c] Subject 10: s_T $P < .001$.

( ) = without disparate pair of duplicates for Subject 10 (see Table XII).

† = this is the only instance in which there was no significant biologic variation (see Section V E).

TABLE V

Cholesterol Levels Before and During Vitamin B_{12} Administration in Two Subjects Who Showed Significant Changes

Subject No.	Cholesterol Level in mg. per 100 cc.	Number of Observations: Period I	Period II and III	Period IVB
		27 days	*77 days*	*85 days*
7	>220	10	14	1
	200–220	6	6	15
	<200	0	0	10
Total		16	20	26
		27 days	*73 days*	*92 days*
9	>270	8	9	1
	240–270	8	9	16
	<240	0	0	9
Total		16	18	26

TABLE VI

Vitamin B_{12} Levels in the Blood Serum of Six Healthy Subjects Before and After B_{12} Administration

	Serum Sample		Vitamin B_{12} Levels in $\mu\mu$g. per cc.						
	Date	No.	Subject No.						
			3	6	7	8	9	10	Mean
Period III. Control period before vitamin B_{12}	Jan. 14–16	1	222	163	420	93	426	181	250.8
	Feb. 6–11[1]	2a	192	82	58	70	257	198	142.8
		2b	192	65	—	70	216	192	147.0
		Mean 2	192	73.5	58	70	236.5	195	137.5[3]
		Mean 1 & 2	207.0	118.2	239.0	81.5	331.2	188.0	194.2
Period IVB. Vitamin B_{12} 500 micrograms per day	March 4–6	3	227	280	297	204	233	297	256.3
	May 20[2]–June 5	4a	262	233	367	210	623	198	315.5
		4b	233	210	327	227	589	187	295.5
		Mean 4	247.5	221.5	347.0	218.5	606.0	192.5	305.5
		Mean 3 & 4	237.2	250.8	322.0	211.2	419.5	244.8	280.9
Mean 3 & 4 / Mean 1 & 2			1.15	2.12	1.35	2.59	1.27	1.30	1.45

[1] Duplicate tests carried out by technician voluntarily.
[2] Duplicate tests performed blind.
[3] Horizontal mean.

The fact that his later B_{12} blood level was considerably lower may explain the fact that the decrease in cholesterol level was not maintained. Although a less striking example, Subject 10 exhibited the same trend with a low mean cholesterol value of 162.5 mg. per 100 cc. on the day when his vitamin B_{12} blood level was highest, namely, 297 $\mu\mu$g. per cc. (Fig. 4). We may speculate whether, if Subject 8 had achieved a higher vitamin B_{12} blood level he might also have had a significant decrease in his cholesterol level. As it was, his blood level was never found to be above 227 $\mu\mu$g. per cc., which is well below the mean of 269.8 for healthy young adults mentioned above.

3. *Effect on body weight, blood pressure and heart rate.* Several subjects showed significant changes in body weight when the mean weight for Periods I, II and III was compared with the mean weight for Period IVA and B combined. However, as shown in Table VII and in Figures 1–4, there was no uniformity in the *direction* of these significant mean changes. Thus, while Subjects 6 and 8 showed a mean *gain* of 4.3 and 4.6 lbs. respectively, Subjects 3, 9, and 10 showed a mean *loss* of 1.4, 1.7, and 1.9 lbs. respectively.[13] Subject 7 was the only one on vitamin B_{12} who showed no significant change in body weight. Accordingly, there is nothing to point toward any single immediate effect of vitamin B_{12} administration upon the body weight of these healthy young adults. However, it is noteworthy that the two subjects (6 and 8) who gained weight were among those with the highest cholesterol levels to begin with and showed the largest percentage increase in vitamin B_{12} level (112 per cent and 159 per cent respectively). Examination of the circulatory measurements before and after vitamin B_{12} administration showed that Subject 3 had a significant mean rise in systolic pressure from 124.2 to 132.0 mm. of Hg., and Subject 6 showed a similar rise, from 125.1 to 134.1 mm. of Hg. Subject 9 was the only man who showed a significant rise in diastolic pressure from 73.1 to 79.3 mm. of Hg. There were no significant changes in the mean heart rates of these subjects.

4. *Effect on total variabiltiy and laboratory reproducibility of cholesterol.* Subjects 3, 6, 7, 8, and 9 had no significant difference in total variability (s_T) for Period IVB compared with the control period. Subject 10 showed a significantly larger s_T ($P < .001$) during vitamin B_{12} administration than during the control period. (Subject 10's variability in Period IVB is further considered in the Discussion and Table XII.)

Comparison of the technical error of measurement (s_e) during Period IVB with that of the control period showed no uniform effect of vitamin B_{12} administration on laboratory reproducibility. Subjects 7, 8 and 9 showed no significant differences. In Subjects 3 and 6 the s_e was significantly smaller during Period IVB while in Subject 10 it was significantly larger only when the ques-

[13] Subject 10's weight loss actually began well before vitamin B_{12} administration.

TABLE VII

Summary of All Data per Subject: Cholesterol, Body Weight, Systolic Pressure, Diastolic Pressure and Heart Rate

Subject No.	N[1]	Cholesterol (mg. per 100 cc.)[2]						Body Weight (lbs.)[2]						
		Period					Grand Mean	Period					Grand Mean	Range
		I	II	III	IVA[4]	IVB		I	II	III	IVA[4]	IVB		
1	13	176.9	182.3	—	—	—	179.0	119.9[a]	118.5[a]	—	—	—	119.3[a]	117.8–121.0
2	16	185.1	194.5	186.5[a]	—	—	189.3	165.8[a]	172.4[a]	172.4[a]	—	—	169.1	164.0–174.8
3	35	211.5	225.8	201.0	183.8	213.3	212.6[a]	150.5[g]	149.3[g]	150.2[g]	149.1[g]	148.6[g]	149.4	147.0–152.0
4	15	197.5	187.4	—	—	—	192.8	170.0	169.8	—	—	—	169.9	167.0–173.0
5	14	183.6	186.8	—	—	—	185.0	165.2	166.0	—	—	—	165.5	163.5–167.0
11	21	209.8	211.3	214.9	—	—	211.4	179.3[f]	181.3[f]	181.3	—	—	180.5	178.0–183.0
6	28	297.3	315.5	315.8	327.5	303.0	306.7	187.6[i]	188.5[i]	187.7[i]	190.4[i]	192.6[i]	189.7	185.5–197.0
7	33	227.9	233.8	230.6	213.2	200.8	217.5	175.3	177.7	180.7	181.8	178.1	178.1	174.0–183.5
8	32	265.0	283.0	244.0	277.8	243.4	256.0	164.8[k]	165.0[k]	166.6[k]	167.0[k]	170.5[k]	167.4[a]	163.2–172.2
9	32	268.8	269.8	278.2	277.5	245.0	261.2	185.8[l]	187.9[l]	185.3[l]	184.1[l]	184.5[l]	185.3	181.2–190.2
10	27	190.5	200.0	183.8	184.8	184.4	186.7	163.5[n]	161.0[g, n]	161.0[n]	160.8[n]	160.5[n]	161.5	159.0–165.0

Subject No.	N[1]	Systolic Pressure (mm. Hg)[2]							Diastolic Pressure (mm. Hg)[2]						
		Period					Grand Mean	Range	Period					Grand Mean	Range
		I	II	III	IVA[4]	IVB			I	II	III	IVA[4]	IVB		
1	13	121.7	117.4	—	—	—	119.9[g]	108–142	73.3	71.4	—	—	—	72.5[g]	62–88
2	16	137.0	139.0	142.0[a]	—	—	138.2	126–148	80.8	86.0	65.0[a]	—	—	82.1	60–108
3	35	125.1[h]	122.4[h]	125.6[h]	135.0[h]	131.5[h]	127.3	115–150	61.1[d]	71.2[d]	64.6	71.0	68.1	66.9	45–86
4	15	127.6	133.6	—	—	—	130.4	122–145	86.4	79.0	—	—	—	82.9	70–100
5	14	130.1	129.7	—	—	—	129.9	118–142	74.6	74.0	—	—	—	74.4	68–82
11	21	123.1	121.8	126.5	—	—	123.2	110–138	71.2	76.9	76.0	—	—	74.6	62–86
6	28	126.6[j]	123.8[j]	123.0[j]	129.0[j]	135.1[j]	128.9	115–150	77.9	72.0	79.0	84.5	80.2	78.2	66–90
7	33	126.6	134.0	131.0	129.0	128.0	129.0	114–144	78.2	82.0	75.0	77.0	81.5	79.3	68–90
8	32	122.1	126.5	129.6	127.5	131.8	128.1	110–145	77.4	73.8	74.8	87.0	78.2	77.4	66–90
9	32	119.0	130.5	124.8	125.0	125.8	124.5	110–142	69.2[m]	76.5[m]	76.6[m]	75.0[m]	80.0[m]	76.0	58–100
10	27	119.6	112.0[g]	123.5	125.0	122.2	121.4	112–136	69.6	73.0[g]	69.0	71.0	72.1	70.8	62–82

TABLE VII—*Continued*

Subject No.	N[1]	Heart Rate (Beats per min.)[2]						
		Period					Grand Mean	Range
		I	II	III	IVA[4]	IVB		
1	13	114.3[b]	101.0[b]	—	—	—	108.8[5]	92–128
2	16	72.8	78.3	80.0[6]	—	—	75.5[5]	62–100
3	35	78.2	77.2	82.4	82.0	82.0	80.1	60–96
4	15	80.9	81.0	—	—	—	80.9	72–90
5	14	77.8[e]	71.2[e]	—	—	—	75.2[5]	68–84
11	21	85.9	83.9	102.0	—	—	87.4[5]	72–108
6	28	83.2	82.4	78.7	80.0	83.2	82.4	68–104
7	33	71.5	66.8	75.7	63.0	70.4	70.8[5]	56–92
8	32	85.0	88.0	89.2	80.0[5]	92.9	89.2[5]	76–108
9	32	84.0	91.8	94.0	84.0[6]	89.8	89.1[5]	72–112
10	27	67.1	68.0[6]	67.8	67.0	67.3	67.3	58–84

Statistical Comparisons for body weight and circulatory measurements: (See Tables III and IV and Sections I C 3 and II C 1 for significant cholesterol comparisons).

A. Mean of Period I versus mean of Period II

a $P = .02$
b $.02 < P < .05$
c $P < .001$
d $.02 < P < .05$
e $P = .05$
f $.001 < P < .01$

B. Mean of Period I, II and III combined versus mean of Period IVA and B combined.

g	$.001 < P < .01$	150.0 vs 148.6
h	$.001 < P < .01$	124.2 vs 132.0
i	$P < .001$	187.9 vs 192.2
j	$.01 < P < .02$	123.1 vs 134.1
k	$P < .001$	165.4 vs 170.0
l	$P = .05$	186.1 vs 184.4
m	$.02 < P < .05$	73.1 vs 79.3
n	$P < .001$	162.5 vs 160.6

C. All other comparisons for body weight, blood pressure and heart rate showed no significant differences.

[1] Total number of observations on different days, distributed over the indicated periods.

[2] Cholesterol data are included here to promote easy comparison with the other data. See Tables III, IV, and XI for detailed analysis. In this table N for cholesterol refers to the number of *pairs* of *duplicate tests* rather than the number of *individual samples*, which would be twice as many (as in Table XI).

[3] These measurements, unlike cholesterol, were not performed in duplicate.

[4] In period IVA there were always two observations unless otherwise indicated.

[5] There was one less observation for this measurement in this subject than is indicated under N.

[6] There was only one observation for this subject in this period.

tionable large difference of 69 mg. per 100 cc. was included (see Discussion and Table XII).

III. RELATIONSHIP OF CHOLESTEROL LEVELS TO ACUTE INFECTIONS

These studies were carried out in 1957–58, the year of the Asian influenza epidemic. Despite some morbidity among their classmates, none of our subjects had any febrile illness while under observation until near the beginning of Christmas vacation. The eight subsequent illnesses in seven subjects are listed in order of occurrence, together with the pertinent cholesterol levels, in Table VIII. All of the serum samples for the postinfectional cholesterol levels were obtained in our research laboratory in the routine manner after the subjects were ambulatory and had returned to classes. Although the number of observations is too small and the data too heterogeneous as to timing and type of illness for satisfactory statistical analysis, several points are worthy of comment:

TABLE VIII

The Effect of Acute Infection Upon Cholesterol Level

Subject	Date	Clinical State	Cholesterol Level		
			I	II	Mean
2	10/1–10/24		185.5[1]	184.6[1]	185.0
	12/12		193	193	193.0
	12/16–12/20	Anorexia 3 days; fever, nausea, vomiting 1 day			
	1/3		*180*	*193*	*186.5*
6A	10/8–10/31		297.8[1]	296.9[1]	297.4
	12/12		325	325	325.0
	12/22–12/27	Cold, sore throat, slight fever, cough			
	1/9	Still coughing a little	*325*	*305*	*315.0*
11	1/28–2/11		206.6[2]	209.4[2]	208.0
	2/13		233	240	236.5
	2/18	"Felt something coming on"	225	233	229.0
	2/19–2/20	Fever, chills, sore throat, lassitude			
	2/21		*175*	*170*	*172.5*
	3/4		218	218	218.0
8	10/31–11/26		264.9[1]	265.1[1]	265.0
	3/21		295	270	282.5
	3/22	Muscle and joint pains			
	3/23–3/24	T. 102°, tightness in chest, weak, nauseated			
	3/25	Still weak, eyes ache	*180*	*180*	*180.0*
	4/2	Much better	240	240	240.0
10	10/31–12/5		188.8[1]	192.2[1]	190.5
	4/2		186	255	220.5
	4/9		212	218	215
	4/16–4/18	Fever, vomiting, diarrhoea			
	4/21		*135*	*142*	*138.5*
	4/29		165	154	159.5
	5/9		180	170	175.0
7	10/31–11/26		229.0[1]	226.8[1]	227.9
	4/22		212	212	212.0
	4/29		193	200	196.5
	5/8		218	225	221.5
	5/9	Head cold			
	5/10	"Bronchitis", T. 101°			
	5/11	No fever			
	5/13	"Head stopped up", some cough, weak	*200*	*186*	*193.0*
	5/20	Slight cough, better	186	186	186.0
	5/27		175	193	184.0
6B	10/8–10/31	See 6A			
	4/17		305	295	300.0
	5/1		335	335	335.0
	5/13–5/14	Nasopharyngitis, probably no fever			

TABLE VIII—*Continued*

Subject	Date	Clinical State	Cholesterol Level		
			I	II	Mean
	5/15		*325*	*335*	*330.0*
	5/22		295	278	286.5
9	10/31–11/26		270.8[1]	266.8[1]	268.8
	5/1		255	263	259.0
	5/8		233	248	240.5
	5/16		287	263	275.0
	5/18–5/19	Nasopharyngitis, tracheitis			
	5/22	Coryza, T. 97.8°, weakness, malaise	*240*	*233*	*236.5*
	5/29		233	255	244.0

The infections are tabulated in chronological order for the academic year 1957–1958.
Subject 6 had two infections, A and B. Control values: 1 = mean of 8 tests; 2 = mean of 5 tests.
Where no comment is made, subject was in good health.

1. No cholesterol levels were obtained in close temporal relationship to the first two illnesses; levels taken two weeks after the infections were over were in no way unusual (Subjects 2 and 6A).

2. The next three illnesses, in February, March and April, were influenza-like infections (Subjects 11, 8 and 10). Each of these three men showed a striking decrease in mean cholesterol level immediately after the attack amounting to −35.5, −85.0 and −52.0 mg. per 100 cc. respectively in comparison with the control value. (See arrows on individual graphs, Figs. 1 and 4.) In fact, in each instance, the mean of the duplicate levels obtained just after the acute illness had subsided was the lowest recorded for that individual throughout the period of observation. (Subject 11, lowest of 21 duplicate tests; Subject 8, lowest of 32 duplicate tests; Subject 10, lowest of 27 duplicate tests.)

3. The last three infections occurred in May, with little or no fever, and were more like variants of the common cold (Subjects 7, 6 and 9). They were not associated with any sharp decline in cholesterol level (Figs. 2 and 3).

Subject 11, the only one whose infection occurred while biweekly cholesterol determinations were in progress, showed an apparent *rise* in cholesterol level just before the infection began. Although the evidence is only suggestive, Subjects 6 (A), 8 and 10 also had cholesterol levels in the upper portion of their ranges just prior to their infections.

IV. LABORATORY REPRODUCIBILITY

In this 1957–1958 study, a total of 530 cholesterol determinations were carried out by the modified Bloor method on 11 subjects with multiple pairs of blind duplicate tests per subject, making a total of 265 pairs of duplicate tests in all.

TABLE IX

Agreement of Duplicate Tests from the Same Serum Sample at Different Cholesterol Levels: 1957–1958 Series

First Cholesterol Level[1] Between	Number of Duplicate Tests	Technical Error of Measurement s_e[2]	Percentage of Duplicate Tests at Each Level Showing Differences of		
			0 to ±9 mg. %	±10 to ±19 mg. %	±20 mg. % or more
mg. %		mg. %	%	%	%
150–199	79 (78)	10.0 (8.5)	64.6	25.3	10.1
200–249	111	8.5	71.2	18.0	10.8
250–299	53	13.6	54.7	18.9	26.4
300–349	19	10.4	21.1	57.9	21.1
All data 100–399	265 (264)	10.3 (9.9)	62.3	23.0	14.7
			0 mg. %	±1 to ±25 mg. %	±26 or more mg. %
			%	%	%
All data 100–399	265		29.4	61.5	9.1

[1] Which one of the duplicate tests was "first" was determined entirely by chance.

[2] The technical error of measurement is

$$s_e = \sqrt{\frac{\Sigma d^2}{2k}}$$ in which d = difference between duplicate determinations and k = number of pairs.

( ) = excluding the | Cholesterol 2 − Cholesterol 1 | difference of 69 mg. per 100 cc. for Subject 10 from the s_e estimate only.

A. Agreement of duplicate tests

Table IX shows the agreement of duplicate tests from the same serum sample at different first cholesterol levels as well as for all the data combined. The percentage of duplicate tests at each level and for all the data for the three classes of numerical differences (0 to ±9, ±10 to ±19, ±20 mg. per 100 cc. or more) is substantially like that of the combined 1955 and 1956 laboratory reproducibility studies already reported[14] (2).

In the 1957–58 series, we found 29.4 per cent of all the duplicates, or a total of 78 pairs, in exact agreement, while 90.9 per cent of the 265 duplicate tests had absolute differences of 25 mg. per 100 cc. or less. Table IX shows that as the first cholesterol level goes up, there is a decrease in the percentage of small differences (only 21.1 per cent of the differences for first cholesterol levels between 300–349 mg. per 100 cc. lay between 0 to ±9 mg. per 100 cc.) and an increase in the percentage of largest differences (26.4 per cent and 21.1 per

[14] 1955 series had 76 subjects with 76 duplicate tests
1956 series had 72 subjects with 72 duplicate tests

cent of the differences were ±20 mg. per 100 cc. or more for first cholesterol levels between 250–299 and 300–349 mg. per 100 cc. respectively).

B. Technical error of measurement

The technical error of measurement in milligrams per 100 cc. (s_e), another measure of duplicate reproducibility, was 10.3 mg. per 100 cc. for all the data, which is in excellent agreement with the figure of 10.7 mg. per 100 cc. for the combined 1955 and 1956 series. Subject 10 had a difference of 69 mg. per 100 cc. for one pair of duplicates, the largest difference among the 265 pairs. As seen in Figure 4, Subject 10 usually showed very little difference between the duplicate determinations, with twelve out of his twenty-seven duplicate tests being in exact agreement. Nevertheless, it was decided not to discard this data, but instead to compare the s_e results when that particular difference was and was not excluded from the calculations. Even with the removal of Subject 10's disparate pair of duplicates, there is no sharp evidence of constant rise in technical error of measurement with rise in cholesterol level for the range of first cholesterol levels studied here, but there is a suggestion that an overall rise in technical error with rise in cholesterol level occurs, using 250 mg. per 100 cc. as a breakpoint.[15]

C. The effect of freezing blood serum on the technical error of measurement

As a by-product of the main purposes of this paper, we were able to examine the effect of freezing blood serum upon the s_e estimates, since part of the cholesterol determinations were performed on frozen and part on fresh serum. In the fresh samples, the chemical determination of cholesterol was performed the day the blood was drawn. Frozen samples were refrigerated a varying number of days before evaluation. Table X shows the s_e values for all the 265 pairs of duplicate serum samples collected from September 25, 1957 through June 5, 1958 classified as to: (1) the frozen or fresh state, (2) first cholesterol level and (3) two time periods of roughly equal length. Estimates of s_e for 142 frozen and 117 fresh samples for all the data are in excellent agreement, 10.0 and 10.3 mg. per 100 cc. respectively. The two time periods yield very much the same estimates of s_e for all the data: 10.5 and 10.1 mg. per 100 cc. respectively. Estimates of s_e for frozen and fresh samples for the two time periods are not markedly different. Estimates of s_e for frozen and fresh samples by first cholesterol level are in general agreement.[16] Finally, frozen and fresh data per man were studied for differences in s_e estimates on seven of the 11 subjects who had enough data to

[15] For first cholesterol levels between 150–249 mg. per 100 cc. s_e was computed to be 9.2 (8.5) mg. per 100 cc., while for first cholesterol levels between 250–349 mg. per 100 cc. s_e was computed to be 12.8 mg. per 100 cc. These s_e's are significantly different.

[16] However frozen s_e = 9.6 and fresh s_e = 6.2, with Subject 10's large difference of 69 excluded, are significantly different, $.005 < P < .01$.

TABLE X

Laboratory Reproducibility of Total Serum Cholesterol Values from Fresh and Frozen Serum Samples, as Measured by s_e Estimates

Grouping of Data	Frozen		Fresh		Frozen, Fresh, and Unknown	
	Number of pairs of duplicate tests	s_e	Number of pairs of duplicate tests	s_e	Number of pairs of duplicate tests	s_e
		mg. %		mg. %		mg. %
A. By first cholesterol level[1]						
150–199	49	9.6	28 (27)	11.0 (6.2)	79 (78)	10.0 (8.5)
200–249	55	8.7	55	8.5	111	8.5
250–299	25	12.5	25	13.2	53	13.6
300–349	11	11.5	8	8.7	19	10.4
B. All data[2]						
100–399 and 9/25/57–6/5/58	142	10.0	117 (116)	10.3 (9.3)	265 (264)	10.3 (9.9)
C. By date of serum sample						
9/25/57–1/23/58, 121 days	71	10.4	71[3]	9.8[3]	148	10.5
1/28/58–6/5/58, 129 days	71	9.6	46 (45)	10.9 (8.3)	117 (116)	10.1 (9.1)

[1] Which one of the duplicate tests was "first" was determined entirely by chance.

[2] Two first cholesterol levels fell below 150 mg. per 100 cc., one fresh (Subject 10), and one frozen (Subject 1) while one fell above 349 mg. per 100 cc. (Subject 6) and was frozen.

[3] These sera were fresh with rare exceptions.

( ) = excluding the | Cholesterol 2 − Cholesterol 1 | difference of 69 mg. per 100 cc. for Subject 10.

warrant such comparisons. Again we found no evidence that frozen and fresh samples yield laboratory results of different reproducibility. Accordingly, there is no reason to believe that freezing the blood serum has affected the laboratory reproducibility of the total serum cholesterol results.

V. THE NATURE OF INDIVIDUAL CHOLESTEROL VARIABILITY

Table XI gives the grand summary of all cholesterol data per subject for the 11 subjects, who have been grouped by the magnitude of their grand cholesterol means. The 11 subjects were observed continuously for periods ranging from 58 to 233 days, or roughly 2 to 8 months. As many as 54 to 68 single cholesterol determinations per individual were made on six subjects. The grand means run from a low of 179 to a high of 307 mgs. per 100 cc., a wide range of cholesterol types, with single readings ranging from 135–350 mgs. per 100 cc.

For the six individuals who were followed during Period IV while on vitamin B_{12}, the grand summary cholesterol data is first given *without* the Period IV data being included, so that all 11 subjects can be compared on a similar basis as to time observed. The total data for the same six subjects (Periods I–IV inclusive) are then given in the second part of Table XI. It may be noted that the "stop smoking" experiment data on Subjects 1–5 and 11 are

TABLE XI

Grand Summary of Cholesterol Data per Subject

Periods Summarized	Group	Subject No.	Total Time Observed in Days	Cholesterol in mgs. per 100 cc.						
				Total N	Grand Mean	Range	Size of Range	s_T	s_e	s_b
I, II and III[1, 2]	A. Grand Mean <200 mg. per 100 cc.	1	58	26	179.0	148–212	64	17.5	7.3	16.3
		5	69	28	185.0	154–212	58	15.4	7.0	13.9
		10	99	26	189.2	175–212	37	10.8	6.5	8.8
		2	95	32	189.3	170–218	48	12.4	5.8	11.1
		4	76	30	192.8	165–233	68	18.0	13.0	12.7
	B. Grand Mean 200–249 mg. per 100 cc.	11	121	42	211.4	170–255	85	19.0	8.9	17.0
		3	122	40	215.1	175–263	88	21.1	13.4	16.5
		7	104	36	230.1	200–263	63	15.6	7.9	13.6
	C. Grand Mean 250 mg. per 100 cc. or more	8	99	34	263.1	218–345	127	27.4	13.3	24.4
		9	100	34	271.8	240–315	75	17.4	10.1	14.4
		6	122	32	306.5	263–350	87	24.2	13.3	20.6
I–IV[3] inclusive	A. Grand Mean <200 mg. per 100 cc.	10	218	54	186.7	135–255	120	18.8	11.0 (5.8)	—
	B. Grand Mean 200–249 mg. per 100 cc.	3	233	68	212.6	170–263	93	20.3	10.8	—
		7	209	66	217.5	170–263	93	20.4	7.4	—
	C. Grand Mean 250 mg. per 100 cc. or more	8	204	64	256.0	180–345	165	30.1	13.0	—
		9	211	64	261.2	212–315	103	22.7	11.4	—
		6	227	56	306.7	233–350	117	27.2	11.3	—

[1] The total number of cholesterol determinations for all 11 subjects was 530. Range: 135–350 mg. per 100 cc.

Total length of time observed in days as in Table III.

Total N = total number of cholesterol determinations. The number of pairs of duplicate tests from which s_e is derived for each subject is one-half of the total N per subject.

[1] Subjects 1, 4 and 5 not observed in Period III.

[2] The data on the six subjects in the Vitamin B_{12} experiment (Period IV) were summarized both *with* and *without* the Period IV data: a. for purposes of comparison with the other subjects. b. because vitamin B_{12} administration appeared to affect cholesterol levels, thus introducing another factor in addition to those producing normal biologic variation.

( ) = without disparate pair of duplicates for Subject 10 (see Table XII).

pooled with their other cholesterol data, as there was no clear evidence that the experiment had any effect on cholesterol level.

A. The effect of cholesterol level on total variability

The 11 subjects present very different pictures of variability about their own cholesterol levels as can be evidenced by a three-way look at the "size of range" and s_T columns of Table XI and at the individual charts themselves (Figs. 1–4). The range size runs from 37 to 165 mgs. per 100 cc. for these subjects and is not

closely associated with the total time observed in days. For example, when Subjects 10, 2, 7, 9, and 8 were observed a total time of 99, 95, 104, 100 and 99 days respectively in Periods I–III they had the very different range sizes of 37, 48, 63, 75, and 127 mgs. per 100 cc. respectively. In general, the s_T appears to increase step-wise for the three grand mean groups.

B. Individual cholesterol variability

The individual charts show most strikingly how different many of the subjects are in their cholesterol variability (Figs. 1–4). In Group A, Subjects 2 and 10[17] have very close-knit individual charts for periods of 95 and 99 days respectively (Figs. 1, 4, and 5A). In contrast, the individual chart of Subject 8,[17] also observed for a period of 99 days, shows a tremendous range, with large fluctuations between successive cholesterol values (Figs. 4 and 5B).

There are many points of contrast both between individuals with the *same* grand mean level and between individuals with *different* grand mean levels. Note for Group A in Table XI that Subjects 1, 5, and 4, although observed over shorter periods of time than Subjects 2 and 10,[17] have larger range sizes and s_T estimates. In Group B, Subjects 11 and 3,[17] observed 121 and 122 days respectively, have similar estimates for the grand mean, "size of range" and s_T. On the other hand, Subjects 8[17] and 9[17] of Group C, observed 99 and 100 days, differed considerably in cholesterol variability. Subjects 1 of Group A and 9[17] of Group C, with very different cholesterol levels, showed the same degree of cholesterol variability over 58 and 100 days respectively; Subject 1 was the only female subject. Subjects 3[17] of Group B and 6[17] of Group C, observed 122 days each with very different cholesterol levels, had the same degree of cholesterol variability.

C. Cyclical trends

Cyclical trends in cholesterol levels have been observed by others (19, 20). However, among our subjects only Subject 3, studied for 233 days, may possibly be said to show cyclical changes in his individual chart (Fig. 1). The other five subjects shown in the same figure were probably not followed long enough to show such trends, while the vitamin B_{12} experiment may have modified the appearance of cyclical changes in Subjects 6–10. Our data on this important point are therefore inconclusive.

D. The effect of cholesterol level on technical error of measurement

A look at the s_e column of Table XI gives the general impression that the individuals with the largest grand means, and consequently with more single readings above 250 mgs. per 100 cc., consistently had the largest estimates of

[17] Excluding the data from Period IV.

FIG. 5. Contrasting patterns of cholesterol level and variability over a period of sixteen weeks: A. Pattern of cholesterol stability as shown by Subject 10. B. Pattern of cholesterol lability as shown by Subject 8. Each dot represents the mean of two determinations from the same serum sample. The solid line indicates the mean and the broken line the range for the eight determinations carried out during the first four weeks. (From Thomas, C. B.: Md. State Med. J. **8**: 2, 1959. By permission of the Maryland State Medical Journal.)

s_e per man. Conversely, some of the individuals in Group A with the smallest grand means had the smallest estimates of s_e per man (see Subject 2 in particular). This is in keeping with the conclusions reached in Section IV of this paper.

E. Biologic variability

The analysis of variance makes it possible to obtain a numerical expression for the "biologic variation" per man, s_b, separated out from his total cholesterol variability, s_T, which includes the s_e component of variability as well as the s_b (21, 22). Estimates of s_b were obtained for all eleven subjects for the data from Periods I, II, and III only, and these were all of significant amounts. There were well-marked individual differences in the degree of biologic variation within each group, particularly in Groups A and C. It is interesting that Subject 9, observed 100 days with a mean of 271.8 mg. per 100 cc., had less biologic variation than Subject 1, a woman, observed only 58 days with a mean of 179.0 mg. per 100 cc. By and large, the biologic variation seems to rise with grand mean level.

Systematic study by means of F ratios from analysis of variance tables of the 29[18] separate person-time periods in Table III and of the 14 separate person-time periods in Table IV showed that in all but nine instances in Table III and one instance in Table IV the biologic variation was significant. The lack of a significant amount of biologic variation in these ten instances may be interpreted in three different ways:

1. The s_b was truly negligible for the periods in question and therefore the differences between the means of duplicate cholesterol determinations (day to day variation) were solely attributable to the size of the s_e. (Subject 10 may illustrate this in Period III over a span of 31 days. See Fig. 4.)

2. The s_e was so large, (i.e., larger than the average s_e value associated with the mean cholesterol level for the period in question), that the s_b which may have existed was quantitatively obscured. (Subject 4 may illustrate this in Period I over a span of 27 days. See Fig. 1.)

3. The sample size was so small in some of the periods, that the s_b which may have existed was not statistically significant.

In conclusion it would appear that, within the limitations of this small series of 11 subjects, the s_T, s_e, and s_b, all increase with level.

VI. DISCUSSION

The outstanding impression gained after intensive study of the cholesterol levels of this group of 11 medical students over a period of months is that striking innate individual differences exist, so that one needs to be extremely cautious in generalizing about the "normal" behaviour of serum cholesterol.

[18] Period II for Subject 10 and Period III for Subject 2 could not be studied for biologic variability because there was only one pair of observations in duplicate in each instance.

The preliminary study of the effect of stopping smoking on cholesterol levels yielded no direct evidence to answer the primary problem, namely, will high serum cholesterol levels decrease significantly after stopping smoking? Two unforeseen difficulties arose which defeated our original purposes. First, no subject with a high serum cholesterol level would volunteer to stop smoking. Second, of those with lower cholesterol levels who did volunteer to stop smoking, several found it difficult or impossible to stop for more than a very short time. Perhaps because of the constant pressure and tension in a medical student's life, none of our subjects was able to carry out the experiment over a period of more than 8 weeks. It is quite interesting that Subject 4, the only one with a *decrease* in mean cholesterol among the SS subjects (or the controls), had smoked the longest of the SS subjects, 7¼ years, and also the most, 40 cigarettes a day, which is 2 to 4 times the amount of the other SS subjects. If we assume that seasonal pressures were causing all the other subjects, both SS and controls, to have a rise in mean cholesterol in Period II, then Subject 4's small decrease in cholesterol at that time might represent a significant decrease counteracted in part by the seasonal pressure effect. Accordingly, although we found no significant lowering of mean cholesterol after stopping smoking, it is possible that stopping smoking for several months or longer might bring higher cholesterol levels down. Thus far, we have found no such experiments described in the literature.

Brozek and Keys have, however, reported on changes of body weight in 21 normal middle-aged men who stopped smoking cigarettes of their own accord, as compared with a matched control group who did not stop smoking (23). Their subjects were members of a long term study of approximately 300 business and professional men. Two years after stopping smoking, the experimental group as a whole showed a rise of 8.2 lbs. in body weight in comparison to the control group. The range of weight change for the experimental group was not given, but from the standard deviation it can be inferred that a few individuals showed little or no gain after stopping smoking, and some may have lost weight. Only one of our subjects showed a marked weight gain, in contrast to the majority of theirs, but the duration of our experiment was much shorter and our men were much younger than theirs.

The apparent self-selection in the direction of very low cholesterol levels of those who volunteered to stop smoking should be commented on. Although Subject 6, the man with the highest cholesterol values, was aware of the fact that he is "hypercholesteremic" and that smoking might exert some influence in pushing cholesterol levels toward such high values, he was unwilling or unable to give up smoking long enough to see if his levels would actually come down.

Our studies on the effect of vitamin B_{12} administration on cholesterol levels are so preliminary that definite conclusions cannot be drawn as yet. To our knowledge, this is the first time that such a study has been carried out on

human subjects. The fact that five of the six subjects showed lower cholesterol values while on oral vitamin B_{12} and that two showed a significant fall in mean cholesterol levels certainly suggests that the parallellism observed in experimental animals between hypercholesteremia and low vitamin B_{12} blood levels may hold true in human subjects as well, but all other explanations have not yet been ruled out.[19] The significant fall in mean cholesterol amounted to about 30 mg. per 100 cc. in each instance. A change of this magnitude is not remarkable when one cholesterol determination is compared with another, but when the *average* of 26 serum samples collected over a period of about three months shows that much decrease when compared with the mean for a similar control series, the likelihood of such change occurring by chance alone is less than one in a thousand. Moreover, there was suggestive evidence that another subject with high cholesterol levels was also favorably influenced—Subject 6 showed his lowest value as his blood vitamin B_{12} level approached 300 $\mu\mu$g. per cc. Absorption of vitamin B_{12} from the gastrointestinal tract is known to be variable and relatively inefficient. The fact that only Subject 9 achieved really high blood levels, despite massive doses of vitamin B_{12}, substantiates this. At present we are repeating the experiment, using oral vitamin B_{12} with an adjuvant which promotes absorption.

Our finding that acute infection of the influenzal type was accompanied by a sharp transitory fall in cholesterol levels as determined immediately after or during convalescence is in agreement with the reports of others (4, 5, 25–27). Okey and Boyden, in studies of the variations in the lipid content of the blood in women in relation to the menstrual cycle, were among the first to note that blood cholesterol may be lowered by slight respiratory infections (19). None of their subjects was so incapacitated by these "colds" as to be unable to attend classes. However, the authors pointed out that the degree of lowering of blood cholesterol levels was less than that noted in association with menstruation, and hence was within normal limits of variation. Groen *et al.*, while studying the effects of different diets on the cholesterol levels of healthy subjects, observed that cholesterol tended to fall during or immediately after relatively brief, short infections, designated as "flu" or "acute gastroenteritis, with vomiting and diarrhea", or "sore throat" (4). Another illness, designated by

[19] It is generally agreed that freezing blood serum does not materially affect the total cholesterol content (24). Nevertheless, we scrutinized the patterns of fresh versus frozen data for all of the subjects taking vitamin B_{12} to make sure that the significant fall in mean cholesterol shown by Subjects 7 and 9 was not an artifact. During Period IVB, when *both* showed a fall in cholesterol values, Subject 7 had a preponderance of *fresh* samples (and also in the control period) and Subject 9 a preponderance of *frozen* samples (but a preponderance of fresh samples in his control period). There was nothing in the data of the other subjects to point toward any such explanation of the fall in mean cholesterol of these two subjects at that time.

them as a "cold", was accompanied by less definite lowering of blood cholesterol. They emphasized that there was a marked "rebound" of cholesterol levels during convalescence, and they likened the reaction pattern to infection to that following exertion or emotional tension.

In our study, several mild acute respiratory infections were observed, one of which was accompanied by one day of fever, which, unlike the infections mentioned above, did not seem to affect cholesterol levels, possibly because the infections were milder or the infectious organisms were different. This observation suggests an interesting approach to follow in evaluating the metabolic effects of various microbiological agents associated with acute respiratory infections. Several workers have noted what appears to be a direct relationship between the severity of an infection and the degree of depression of the total serum cholesterol. This whole problem is well reviewed by Bloor in his monograph published in 1943 (27). He considers that the evidence is sufficient to conclude that blood cholesterol is concerned in the reaction of the body to infectious disease. He states that "if cholesterol is concerned in the immunization process, a change in blood cholesterol would be expected at the onset of the disease, together with a compensatory change at convalescence, and the data available indicate that both take place." In this setting, our suggestive finding that there may be an increase of cholesterol level just *before* the clinical onset of infection certainly warrants further investigation.

Acute infections are only one of a number of forms of stress which are said to affect cholesterol levels (4–9). Mental and emotional stress have been reported to elevate serum cholesterol, and our findings on first year medical students before, during and after the final Anatomy examination tend to substantiate this hypothesis (9). During the present study the subjects as a group did not undergo any such highly stressful experience as the first year Anatomy examination. The six subjects in the vitamin B_{12} study were all second year students (Subjects 3 and 6–10). Of the six, only Subject 9 showed a suggestive cholesterol rise in association with the Pharmacology examination, while Subject 7 showed a doubtful rise at the time of the Biostatistics examination (Fig. 3). No rise which might be interpreted as a stress effect was noted among the three subjects who were studied up to or through the final examination period from May 27–29. However, since the present investigation was *not* planned to study the effect of stress at examinations, there are insufficient data and sometimes definite gaps in the series of observations before, during and after a given examination.

Subject 11, the first year student who volunteered to enter the "stop smoking" study in January, was one of the subjects in the class of 1961 who participated in the "stress" study previously reported (9). His cholesterol levels were 240 and 233 mg. per 100 cc. on January 8, 1958 at Anatomy examination, but twenty days later were 205 and 200 mg. per 100 cc., his first determinations on

entering the present study (See Fig. 1). His final Biochemistry examination came during his Period II on March 19; the day before, his cholesterol levels were 200, 193 mg. per 100 cc., while one week later his cholesterol was 255, 248 mg. per 100 cc., the highest values observed for him in our continuous record of 121 days in this study. Accordingly, there appears to be some relationship in his cholesterol pattern with the two examinations in question.

The fact that the laboratory reproducibility studies on 265 pairs of blind duplicate tests on 11 subjects in the 1957–1958 series agree so closely with the 148 pairs of such tests on 148 different subjects in the 1955–1956 series is a source of satisfaction, because it indicates that the laboratory method employed is stable. The s_e was 10.7 mg. per 100 cc. in the former series and 10.3 mg. per 100 cc. in the present series. In both series, over 90 per cent of the pairs of the duplicate tests had absolute differences of 25 mg. per 100 cc. or less (2). The present study confirms our previous statement that the Buell modification of the Bloor method is as reproducible and as accurate as other standard techniques, such as the Schoenheimer-Sperry and the Abell methods, and is entirely suitable for research purposes.

Basing our studies on unaveraged duplicates, we found that while there was not a constant rise in technical error of measurement (s_e) with level of cholesterol, there was a definite suggestion that an overall rise in s_e occurred, using 250 mg. per 100 cc. as a break point.[20] Of even greater interest is the finding that, when subjects were grouped by the grand mean of all cholesterol values per man, the biological variability (in addition to the technical variability) of those with higher values was on the whole greater than of those with lower values. However, at any given grand mean cholesterol level, there seemed to be ***individual*** differences in the tendency to constancy on the one hand or toward fluctuation on the other. It will take a great deal of work to establish whether over long periods the relative constancy of cholesterol level is an individual characteristic truly independent of level and of all the intercurrent episodes of illness or other forms of stress which may affect it.

Subject 10 illustrates some of the complexities of the situation. His data in Period IVB are considerably more variable than in Periods I, II and III combined (Fig. 4). The extreme values of Period IVB, *255* and *135* mg. per 100 cc., were due to one value of a pair of duplicates with a difference of 69 mg. per 100 cc. (*186, 255*) and to one value of a pair of duplicates after an acute infection (*135, 142*). The difference of 69 mg. per 100 cc. was the largest among the 265 pairs of duplicates for all the subjects in this study and came from duplicate

[20] Chandler *et al.* found that fluctuations in total serum cholesterol levels during a 24 hour period were no greater than those attributable to measurement error alone (28). On the basis of this finding, and because in some periods the number of determinations per man was small, we did not average the duplicate determinations. Keeping the duplicates unaveraged tends to enhance the variability about the mean so that the results of statistical tests comparing these means tend to be conservative.

TABLE XII

Effect of Excluding Certain Cholesterol Determinations on the Mean Value and Estimates of Variability for Subject 10 During Period IV B

Total N	Exclusions as in Footnotes	Cholesterol in mg. per 100 cc.		
		Mean ± S.E. Mean	s_T	s_e
20	1, 2	185.4 ± 4.0	18.0	4.2
22	1	188.6 ± 4.8	22.6	15.3
22	2	181.1 ± 4.7	22.0	4.3
23	3	181.4 ± 4.5	21.5	—
24	4	184.4 ± 5.3	25.9	14.7

[1] Excluding post-influenzal pair of duplicate determinations.
[2] Excluding disparate pair of duplicates with difference of 69 mg. per 100 cc.
[3] Excluding the 255 mg. per 100 cc. determination only of the disparate pair of duplicates.
[4] No exclusions.

determinations on a fresh serum sample.[21] There is ample argument for excluding this atypical difference of 69 from estimates of s_e, both for Subject 10 alone and for group data where it might be included (see Tables IV and XI). However, there are two points of argument for *not* excluding the extreme value of 255 mg. per 100 cc. from calculations other than s_e. First, a test of outlying observations was applied to Period IVB data to see if "255" was an outlier in this series of 24 values, and the test was not found to be significant at the 5 per cent level (29). Furthermore, although "255" seems to be too high a value for Subject 10, i.e., not characteristic of his general cholesterol record, it is interesting that the value occurred two weeks before his acute infection and might be associated with its onset. The next highest value in Subject 10's entire series for all periods was 218, which appeared in the next pair of duplicates following the high reading of 255, and occurred one week before the acute infection. Table XII shows Subject 10's Period IVB data with and without his two extreme cholesterol values. The mean level is little affected and s_T is somewhat affected by the various exclusions listed in the footnotes; only s_e values are markedly affected by the exclusion of the disparate pair of duplicates. Comparison of s_T for Period IVB, even without the disparate and post-influenzal values, with the s_T for Periods I, II, and III combined, shows that Period IVB still had a significantly larger amount of variability about the mean level, for whatever reason.

In reporting a much larger series of subjects than ours, the Watkin group, whose s_T was derived somewhat differently from ours, found that "with respect to total cholesterol, it is of interest that s_e increases with level, and that s_T and s_b do not" (20). Several other authors have commented on the question of biologic variation of cholesterol levels. Particularly pertinent to our findings is a statement by Chandler, Lawry, Potee, and Mann, that variability studies extending over a period of time show "that biologic variation (variation corrected for measurement error) also increases with level." (28). In a study of familial hypercholesteremic xanthomatosis by Guravich, published since our

[21] The next highest difference in the entire study was 51 mg. per 100 cc. (Subject 3). Both of these differences are unusually high (see Table IX).

material was collected, he states that although not enough patients have had repeated serum lipid determinations to allow definitive comment on the individual variability of the serum cholesterol, his findings permit the tentative conclusion that some hypercholesteremic subjects show a rather marked constancy and others a considerable degree of lability with respect to their serum cholesterol levels (30).

VII. SUMMARY

1. There are marked individual differences in level and variability of serum cholesterol in healthy young adults living and working in the same environment.

2. An experiment designed to study the effect of stopping smoking on cholesterol levels was inconclusive because of unforeseen difficulties.

3. The volunteers to stop smoking showed an interesting degree of self-selection in the direction of very low cholesterol values when compared with their classmates.

4. The six volunteers, with low cholesterol values to begin with, showed no definite tendency to develop significantly lower cholesterol values during the "stop smoking" experiment, which continued for less than two months.

5. Of these six subjects, only one showed a marked gain in weight beginning soon after stopping smoking.

6. Because hypercholesteremia has been reported to follow vitamin B_{12} deficiency in rats, crystalline vitamin B_{12} was administered orally to six subjects for more than three months in order to observe its effect on cholesterol levels.

7. On oral vitamin B_{12}, two of six subjects showed a significant fall in mean cholesterol levels amounting to about 30 mg. per 100 cc.

8. Oral administration of 1000 micrograms of crystalline vitamin B_{12} every other day did not produce exceptionally high vitamin B_{12} blood levels in the majority of subjects.

9. During vitamin B_{12} administration, the two men who showed the greatest fall in mean cholesterol also attained the highest vitamin B_{12} blood levels among the six experimental subjects.

10. There was no consistent change in body weight, blood pressure or heart rate during vitamin B_{12} administration.

11. Acute infections of the influenzal type were accompanied by a sharp transitory fall in cholesterol levels in three subjects.

12. Laboratory reproducibility studies on 265 pairs of blind duplicate tests on 11 subjects in the 1957–58 series agreed closely with the 148 pairs of such tests on 148 different subjects in the 1955–56 series.

13. While there was not a constant rise in technical error of measurement (s_e) with level of cholesterol, there was a definite suggestion that an overall rise in s_e occurs, using 250 mg. per 100 cc. as a break point.

14. No marked differences in s_e were observed between fresh and frozen

serum at any cholesterol level or during two different time periods of approximately four months each.

15. When subjects were grouped by the grand mean of all cholesterol values per man, the total variability (s_T) and the biological variability (s_b) of those with higher values was on the whole greater than of those with lower values.

16. At any given grand mean cholesterol level there were individual differences in the tendency toward constancy of cholesterol level on the one hand or toward fluctuation on the other.

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ABSTRACTS OF PAPERS PRESENTED BEFORE THE MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY

April 13, 1959

MAINTENANCE OF THE HEART BEAT BY PERFUSION OF THE CORONARY CIRCULATION WITH GASEOUS OXYGEN

DAVID C. SABISTON, JR., JAMES L. TALBERT, LEE H. RILEY, JR. AND ALFRED BLALOCK

The ability of mammalian tissues and organs to utilize oxygen when perfused in the gaseous state is a fundamental problem of considerable physiological importance. Recently it has been shown experimentally by Burns, Robson, and Smith that the isolated rabbit heart will continue to beat when the coronary circulation is perfused with oxygen. The data reported here concern studies on the dog heart with perfusion of oxygen. In the first group of experiments the isolated heart was studied after removal from the chest. The coronary circulation was perfused for a brief period with blood followed by Tyrode's solution. The perfusion was then changed to a mixture of oxygen (95%) and carbon dioxide (5%). While perfused with gas alone the heart continued to beat actively for periods up to eight hours. When no gas was perfused or when the oxygen perfusion was suddenly terminated, cardiac arrest occurred in 8 to 18 minutes.

In a second group of animals the heart was perfused *in situ* with oxygen for periods of 25 minutes or longer with separate perfusion of the cerebral circulation with blood. Under these circumstances the heart continued to beat and it was possible to restore the normal coronary circulation in 9 of 12 animals with spontaneous maintenance of a satisfactory systemic arterial pressure and with survival periods of one to 48 hours.

These studies indicate that the heart may be maintained in an active metabolic and functional state for prolonged periods by the perfusion of gaseous oxygen alone. They also demonstrate that the coronary circulation may be shifted from a liquid to a gaseous state with subsequent return to a liquid medium.

STUDIES ON COLLAGEN-CRYSTAL RELATIONSHIPS IN BONE MATRIX

ROBERT A. ROBINSON

The papers by Dr. Sheldon and Dr. Robinson dealt with electron micrographic studies of bone in ricketic animals. In order to understand the abnormal situation in ricketic bone, Dr. Robinson presented the electron micrographs of normal bone during development showing the relation of the cells to the matrix and of the collagen fibrils of the matrix to the inorganic hydroxyapatite crystals.

Studies of ricketic bone from rats on a low phosphate Vitamin D deficient diet, show that the collagen fibrils produced by the cells appear normal by electron microscopy. Furthermore, the first evidence of crystals appeared in an approximately similar relationship to the a and c minor bands of the collagen fibrils to that observed by Fitton-Jackson in developing embryonic avian bone.

In the discussion it was pointed out that there was a lag of about two hours between earliest crystal formation in the matrix and an intra-peritoneal injection of inorganic phosphate in ricketic rats. In relation to this lag various investigations of nucleation of apatite by collagen was reviewed. The suggestion was made that the cell probably has to process the phosphate and possibly uncover certain reactive groups on the collagen fibrils before apatite crystals appear in the osteoid.

ELECTRON MICROSCOPE OBSERVATIONS ON RICKETS

HUNTINGTON SHELDON

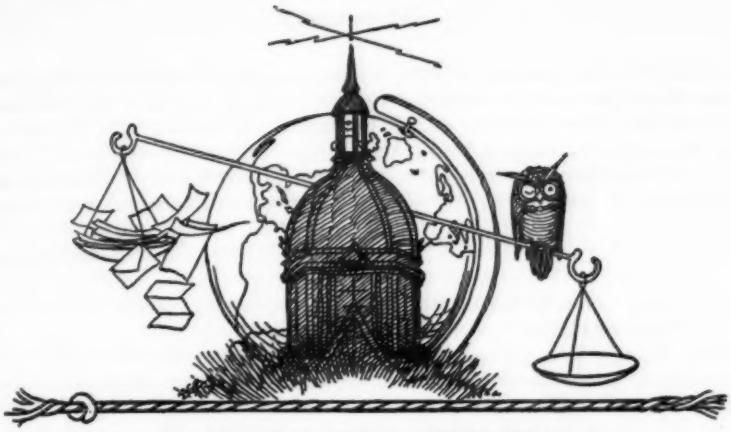
Young rats that are fed a diet low in phosphate rapidly develop the clinical and pathological characteristics of rickets: they fail to gain weight normally, their serum phosphate falls to low levels and they show anatomic lesions which follow the failure to deposit mineral in cartilage and bone. At the end of three weeks on the diet used for these experiments the serum phosphate of experimental animals often is as low as 1.2 milligrams per cent. Observations with the light microscope on undecalcified sections from the tibias of these animals show wide zones of uncalcified eosinophilic bone matrix (osteoid) about the trabeculae, and a lack of calcification in the epiphyseal apparatus. In the electron microscope, sections from the eosinophilic ricketic osteoid show closely packed collagen fibrils that have a periodic structure similar to the banding described in native collagen from rat tail tendon; seven sub-bands have been seen in phosphotungstic acid treated specimens.

At the end of two weeks on the diet, two cc. of an inorganic phosphate solution was administered intraperitoneally to ricketic animals and they were maintained on the rickets-producing diet for varying additional periods up to two weeks. The earliest evidence of mineral deposit in the bone matrix that can be seen in the light microscope after staining with the Von Kossa method is 15 hours after the phosphate is given. But the osteoblasts in the ricketic matrix show large numbers of PAS positive granules in their cytoplasm as early as six hours after the administration of phosphate, before any mineral deposit can be detected. Although I have seen that the acinar cells of the pancreas of ricketic rats often appear less basophilic than the cells in the pancreas of normal controls, and have observed that the intense cytoplasmic basophilia returns within six hours after the administration of phosphate, these changes are not sufficiently reproducible so that I feel any conclusions can be drawn from them at this time.

The electron microscope shows that small crystals appear in the bone and cartilage matrix as early as two hours after the phosphate is given. The PAS positive granules that are seen in the light microscope correspond most closely in the electron microscope to dilatations in the endoplasmic reticulum of the osteoblast cytoplasm. These dilatations in the endoplasmic reticulum have been called cisternae and have been implicated in the synthesis of protein (zymogen) by the pancreatic acinar cell.

Further observations show a matrix substance unlike any previously described matrix of cartilage or bone; this material lies about many osteoblasts in areas where one might expect new matrix to be deposited. This hitherto undescribed matrix material only can be defined at this time as a finely granular aggregate in which suggestions of filaments are seen. A three dimensional reconstruction from sections has not been made, but staining with phosphotungstic acid after sections have been cut (the method of Watson) shows occasional larger fibrils in this amorphous matrix which are recognizable as collagen fibrils by their periodicity. The material within the cisternae of the osteoblast cytoplasm did not stain with phosphotungstic acid, but there is a similarity in appearance between the cisternal contents and the extracellular matrix. Some sections show an apparent continuity between the osteoblast cisternae and the extracellular material which suggests a manner whereby material originating within the basophilic component of the cytoplasm of cells be secreted into the extracellular space.

In the light of these experiments it might be worthwhile looking for further evidence that the administration of inorganic phosphate has a direct effect on the production of bone matrix. It is suggested that low phosphate rickets, like scurvy, may be a systemic disease which affects more than the skeletal system alone.



BOOK REVIEWS

A Short Practice of Surgery, 11th edition. By HAMILTON BAILEY AND MCNEILL LOVE. 1389 pp., $18.00. J. B. Lippincott Co., Philadelphia, Pa.

As the authors indicate, the "Short" Practice of Surgery, is, in fact, a massive one volume text, which is as complete as a one volume surgical text could hope to be. The first edition was published in 1932. Nevertheless there are no "quaint" illustrations, and the text gives evidence of repeated and careful re-writing, rather than the piecemeal engrafting of new material on old, which is so likely to occur in new editions of standard texts. All of the surgical specialties are treated in detail. This very wide coverage, together with the use of a systematic format, aided by many headings and sub-headings, leads to an almost telegraphic style. This is further emphasized by the rather authoritarian and didactic attitude employed in most sections. Nevertheless a fair selection of alternative methods of treatment, or theories of etiology, is usually given. The footnotes, explanatory of eponyms in the text, or providing biographical material relative to authors quoted, are a unique and interesting feature of the book. Unfortunately, this is not accompanied by specific citations from the literature, and none of the chapters has a bibliography.

Some authoritative one volume text of surgery, of all inclusive scope, such as this, should be on every surgeon's desk. This is a sound, authoritative and complete volume and will serve its purpose extremely well.

MARK M. RAVITCH

Surgical Pathology, 2nd edition. By LAUREN V. ACKERMAN in collaboration with HARVEY R. BUTCHER, JR. 1096 pp., $15.00. The C. V. Mosby Co., St. Louis, Mo.

Dr. Lauren V. Ackerman, Professor of Surgical Pathology at Washington University School of Medicine has recently extensively revised his textbook on surgical pathology, the first edition having been published in 1953. The earlier edition became a standard reference book in all pathology laboratories. The new second edition with its large number of superior photographs of diseased organs, gross lesions and photomicrographs of these lesions, will set a new standard of excellence for surgical pathology texts. We pathologists who work primarily in surgical pathology can be highly gratified with Dr. Ackerman's demonstration of what can be accomplished in the field of surgical pathology when the proper facilities are available for the adequate studies of tissues removed at operation. These encompass most of the diseases that one finds in autopsy pathology with the possible exception of

myocardial infarction. Because, in biopsy diagnosis the patient is still alive, the incentive for close clinical-pathological correlation is ever present. To put added weight on this aspect of the text, Dr. Harvey Butcher, a surgeon, edited the book along with Ackerman. The chapter on surgical pathology of the eye has been very ably written by Dr. L. E. Zimmerman of the Armed Forces Institute of Pathology. For the practitioners of the various medical specialties, this book will be very valuable and it will not see its peer for a long time to come.

STANTON L. EVERSOLE

Current Therapy 1959. Edited by HOWARD F. CONN. 781 pp., $12.00. W. B. Saunders Co., Philadelphia, Pa.

Current Therapy is a deservedly popular manual of therapeutics. The 1959 edition is the eleventh in an annual series, and it differs little from its immediate predecessors in format and content. Therapeutic recommendations for a multiplicity of disease entities are set down in a clear and concise manner by recognized authorities. The value of the volume is further enhanced by the supplementary tables of normal laboratory values, commonly encountered toxic agents, and descriptions of currently used pharmaceutical preparations.

One might wonder at the need for annual revisions. This practice can be defended in that it provides for prompt publication of significant therapeutic advances and permits an expression of differing opinions by several authors. Current Therapy, of course, is a "quick reference manual" and does not provide one with insight into the pathophysiology of disease or the pharmacologic basis of therapeutics.

ALBERT H. OWENS, JR.

Long-Term Illness. Management of the Chronically Ill Patient. Edited by MICHAEL G. WOHL. 748 pp., $17.00. W. B. Saunders Co., Philadelphia, Pa.

This book is designed primarily for the practicing physician, medical student and rehabilitation worker, and 80 distinguished physicians collaborate to fulfill the avowed intent of producing a comprehensive survey of the management of the patient with prolonged illness. In this they fail.

Individual chapters vary from fair to excellent—the section on chronic diseases in children seemed very good to one whose knowledge about the subject was scanty—although in all sections the illustrative x-rays reproduce poorly and are uniformly unconvincing. The medical student would obtain a good general survey of the field from this work but the rehabilitation worker would prefer to see more details of specific physical measures necessary in the treatment of individual diseases, and fewer generalizations.

Therapeutic information is brought up to date, but a critical evaluation of newer remedies is usually lacking. In some sections a disproportionate amount of space is used to detail unproved potentially hazardous measures such as the use of nitrogen mustard in the treatment of rheumatoid arthritis. I hope most physicians would wince at the orthopaedic advocacy of a steroid-salicylate preparation as an analgesic for backache.

To have been uniformly successful ln tackling this important subject, the presentation of which bristles with so many difficulties, would have been a great triumph.

VERNA WRIGHT

Nurse-Patient Relationships in Psychiatry, 2nd edition. By HELENA WILLIS RENDER AND M. OLGA WEISS. 319 pp., $5.95. McGraw-Hill Book Co., New York, N. Y.

This volume is a considerably revised version of the first edition which was published ten years ago. It is designed primarily for the student nurse and contains an abundance

of specific, simplified directions to guide the new nurse in working with psychiatric patients. As the title of the text indicates, emphasis is placed on the importance of the nurse's personal relationship with the patient, understanding the patient's behavior, coping with it in the light of its meaning, and the development of therapeutic attitudes. This seems an excellent approach and one which deserves the emphasis given by the authors. It is, however, a difficult orientation to combine with didactic instruction about the "do's and don't's" which are associated with the nurse's reaction and handling of specific behavioral symptoms encountered in psychiatric patients. It is easy to see how the task of mastering these problems frightens and overwhelms many student nurses. The authors discuss and explain, for the nurse, many of the fear reactions observed in the patients. It would be equally useful to discuss in equally organized fashion the anxiety and fear reactions to be expected in the novice psychiatric nurse.

The division of the chapters on nursing care according to specific nursing approaches and clinical diagnostic groupings is somewhat clumsy and artificial. The newly added chapter on rehabilitation is well written and is valuable in classifying, for the student, her relationship to the other members of the hospital therapeutic staff. Giving definition and meaning to the roles of various therapeutic disciplines makes it easier for the student to realize and adjust to her own role in the total treatment effort.

This text has established its usefulness in psychiatric nursing instruction, and this new edition assures its continued value.

WILLIAM D. WHEAT

Clinical Epidemiology. By JOHN R. PAUL. 291 pp., $5.00. The University of Chicago Press, Chicago, Ill.

This book is one of a series in "The Scientist's Library: Biology and Medicine." The purpose of this series is "to provide authoritative information about the growth and status in various areas in such a fashion that the individual books may be read with profit not only by the specialist but also by those whose interests lie in other fields. . . . The authors have been asked to emphasize introductory concepts and problems, and the present status of their subjects and to clarify terminology and methods of approach instead of limiting themselves to detailed accounts of current factual knowledge. The authors have also been asked to assume a common level of scientific competence rather than to attempt popularization of the subject matter."

This is indeed a difficult assignment, and Dr. Paul is to be congratulated for his attainment of so many of the objectives.

In his introduction, Dr. Paul states that the purpose of this small volume is to "introduce this subject (of epidemiology) to doctors or students of medicine, biology or sociology in nontechnical language and with examples they might use." Many of the chapters are based on lectures presented in an elective course called "Clinical Epidemiology" given at Yale University to public health students and to medical students. By clinical epidemiology, Dr. Paul means those aspects of the study of the ecology of disease which are "of most immediate interest and use to physicians." The author's own definition states that epidemiology is "concerned with the circumstances under which diseases occur, where diseases tend to flourish, and where they do not."

There are two fascinating chapters in which Dr. Paul presents the historical development of epidemiology from Ancient Greece to the present. It is of interest that here he recognizes the fact that "the introduction of a critical attitude with regard to statistics . . . was in part responsible for developing the field of epidemiology as a science in its own right and one which put an end to the era when conclusions were drawn and generaliza-

tions put forth which were based on clinical impressions rather than measurements." It is a little difficult to reconcile this admission of the contribution of the statistical approach with the sharp dichotomy between the "statistical epidemiologist" and "clinical epidemiologist" which is stressed throughout this book.

The second part of the book deals with the epidemiologic method and the principles upon which that method is based. Emphasis is placed upon attempting to understand disease processes by looking beyond the patient himself to his family and to the community. There are excellent discussions of Experimental Epidemiology and Geographical Pathology which make most interesting and informative reading. There are chapters dealing with terminology and methodology used in handling morbidity and mortality data, and these should be most helpful to the clinician who is responsible for the basic records on which these data are based.

The third part of the book consists of a discussion of the epidemiologic features of several "sample diseases"—specifically rheumatic fever, coronary occlusion, poliomyelitis, infectious and serum hepatitis, and the arthropod-borne virus encephalitides. These presentations are well done, as one would certainly expect from Dr. Paul. The chapter on coronary occlusion is particularly welcome, since the avowed purpose of the book is "to develop the principle that epidemiology is by no means limited in scope to the concept of infectious disease"; and yet one is hard-pressed to find more than passing reference to the non-infectious diseases elsewhere in the book. Dr. Paul's hopes for the future of epidemiology in other fields are somewhat obscured by this apparent emphasis on infectious disease problems.

Dr. Paul has been a pioneer in the field of serological epidemiology and his brilliant contributions to this field are only briefly touched upon in this book. There is an appendix devoted to "Guides for Planning Serologic Surveys" which is of somewhat dubious value in a book of this sort. The general principles as formulated by Dr. Paul and his associates are certainly sound, but there may be an over-simplification of the problems which arise during the planning and conduct of such surveys. Some might be misled by the offhand statement that "Biostatistical advice is helpful in these matters." One wonders what degree of confidence should be placed on the number of 350 as the "ideal" for sampling a given population of 120,000. What seems even more remarkable is the ability to predetermine the number of samples recommended for each age group without being forced to make certain assumptions about the expected distribution of serum antibodies in these different age groups.

This is one of several new textbooks on epidemiology which have appeared in the past two years. It is becoming evident that there are as many ideas about what should go into a textbook as there are epidemiologists courageous enough to attempt one. Because of the potential usefulness of the epidemiologic method to fields other than public health, Dr. Paul's approach is both worthwhile and welcome. It is highly recommended as a book which presents the principles of epidemiology in a form which will be palatable to an "audience of physicians, medical students, internists, biologists, ecologists, . . . welfare workers and social workers." It might be of less value to the public health worker who is already epidemiologically oriented, but this is not the audience to which Dr. Paul is addressing his remarks.

Certainly there is need to dispel the idea that epidemiology involves primarily the study of epidemics, and this book documents the teaching approach that one of this country's outstanding epidemiologists has used so successfully in dealing with this misconception.

RAYMOND SELTSER

Causation of Cancer. British Medical Bulletin, Vol. 14, No. 2, May, 1958. 196 pp., $4.00. Medical Department, The British Council, London, England.

This number of the British Medical Bulletin is devoted to cancer causation and is dedicated to Sir Ernest Kennaway, one of the pioneers in this subject. The present paper back volume containing the papers of 27 distinguished British contributors is a sequel to Vol. 4, No. 5-6, 1947 which dealt with similar subject matter. Although this present volume contains some chapters the pure clinician will find dull, it should be re-read several times by all who profess to treat cancer.

A thought provoking introduction by Professor Alexander Haddow gives a pertinent review of the subject of cancer and suggests some fascinating and challenging problems ahead.

There are 21 papers which seem to adequately embrace the field of cancerogenesis as it is known today. Chemical, biological, immunology, endocrine, metabolic and physical cancerogenesis are each learnedly discussed. There are some excellent papers on experimental cancerogenesis and a brief resume of the field on occupational cancerogenesis by Dr. M. W. Goldbolt. The latter topic is further discussed by A. L. Walpole and M. H. C. Williams in their paper; Aromatic Amines as Carcinogens in Industry. Five papers are devoted to topics concerning the cancerogenic properties of irradiation.

ROBERT G. CHAMBERS

BOOKS RECEIVED FOR REVIEW

Aids to Medical Treatment, 4th edition. By T. H. CROZIER. 367 pp., $3.75. Bailliere, Tindall and Cox, London, England. The Williams & Wilkins Co., Baltimore, Md., exclusive U. S. agents.

Biosynthesis of Terpenes and Sterols. Edited by G. E. W. WOLSTENHOLME AND CECILIA M. O'CONNOR. 811 pp., $8.75. Little, Brown & Co., Boston, Mass.

Cellular and Humoral Aspects of the Hypersensitive States. A Symposium held at The New York Academy of Medicine. Edited by H. SHERWOOD LAWRENCE. 667 pp., $18.00. Paul B. Hoeber, Inc. Medical Book Dept. of Harper & Bros., New York, N. Y.

Rene Dubos Mirage of Health. Utopias, Progress and Biological Change. Edited by RUTH NANDA ANSHEN. 236 pp., $4.00. Harper & Bros., New York, N. Y.

Fundamentals of Otolaryngology, 3rd edition. By LAWRENCE R. BOIES. 510 pp., $8.00. W. B. Saunders Co., Philadelphia, Pa.

Glaucoma. Transactions of the Third Conference, January 1958. Edited by FRANK W. NEWELL. 272 pp., $5.25. The Josiah Macy, Jr. Foundation Publications, New York, N. Y.

The Kansas Doctor: A Century of Pioneering. By THOMAS N. BONNER. 334 pp., $5.00. University of Kansas Press, Lawrence, Kans.

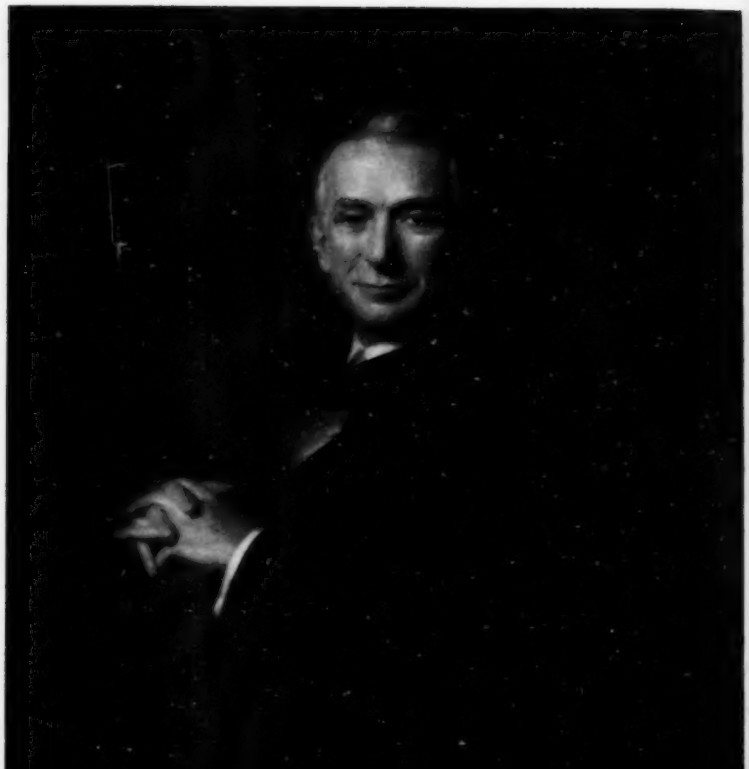
Navy Surgeon. An Autobiography. By REAR ADMIRAL LAMONT PUGH. 459 pp., $5.00. J. B. Lippincott Co., Philadelphia, Pa.

Szondi Test in Diagnosis, Prognosis and Treatment. By LIPOT SZONDI, ULRICH MOSER AND MARVIN W. WEBB. 309 pp., $12.00. J. B. Lippincott Co., Philadelphia, Pa.

Therapeutic Electricity and Ultraviolet Radiation. Vol. IV of Physical Medicine Library. Edited by SIDNEY LICHT. 373 pp., $10.00. Elizabeth Licht, Publisher, New Haven, Conn.

Treatment of Cancer and Allied Diseases. Vol. 3, Tumors of the Head and Neck. Edited by GEORGE T. PACK AND IRVING M. ARIEL. 781 pp., $30.00. Paul B. Hoeber, Inc., Medical Book Dept. of Harper & Bros., New York, N. Y.

Wound Healing and Tissue Repair. Edited by W. BRADFORD PATTERSON. A Report from The Developmental Biology Conference Series, 1956. 83 pp., $2.75. The University of Chicago Press, Chicago, Ill.



ARNOLD R. RICH

REMARKS ON THE OCCASION OF THE PRESENTATION OF A PORTRAIT OF ARNOLD R. RICH TO THE JOHNS HOPKINS UNIVERSITY, FEBRUARY 28, 1959

RICHARD H. FOLLIS, JR.

Dr. Chesney, members and guests of the Association:[1] Two years ago when Dr. Rich's retirement became imminent a group of his classmates and colleagues decided to honor him by having his portrait painted. We felt that this should be a Hopkins family affair; consequently, only his classmates, the students who have been in his teaching groups, former department members, and past and present associates in the Medical School and Hospital were asked to help make this undertaking possible. At that, his Hopkins family was found to be a large one. Such historical details as these bring us to the finished work by Mr. Paul Trebilcock, which is before us this morning. It is for the many who made this portrait a reality that I now speak.

This is not the time to present a biographical sketch of Dr. Rich, to detail his scientific contributions or to enumerate the honors which have been bestowed upon him. Rather, I believe that all who joined in this project would only wish me to express to him why they desired to honor their teacher, counselor and friend.

Many of us met Dr. Rich for the first time in the second year course in pathology. All were stimulated by his lectures on tuberculosis, hypersensitivity and immunity, jaundice, syphilis, blood disturbances and many other subjects. His presentations were lucid and, in particular, full of material drawn from his own experience, usually not what one could find in textbooks, even Dr. MacCallum's. Some were fortunate to be in his teaching group and will doubtless recall afternoon sessions in which he would discuss a variety of subjects and ask stimulating questions that made one think and gave some idea of what disease was all about. It was in the second year, too, that students first attended a teaching exercise which many will remember among the most inspiring hours of medical school days—the CPC. Here Dr. Rich, first in association with Dr. Thayer, then Dr. Hamman, and recently Dr. Harvey, clearly and concisely delineated the clinical course of a given disease and interpreted its final scenes in terms of the findings at the autopsy table. At these conferences Dr. Rich, as a pathologist, was dealing, as he himself has put it, with "the most perfect flower of medical science", pathogenesis.

Those who came into closer contact with Dr. Rich as a result of one or more years on the pathology staff will recall his meticulous care in dissection at the gross conference or his masterly summing up of the completed case

[1] Dr. A. M. Chesney, President of The Johns Hopkins Medical and Surgical Association. The portrait was presented at the biannual meeting of The Johns Hopkins Medical and Surgical Association in 1959.

at the microscopic sessions. And all staff members will remember the stimulus of Journal Club. Here one was amazed and, at times, left with an inferiority complex at his wide knowledge of disease. Many were aroused to pursue research problems following discussions at Journal Club. In his home he was his informal self and, as he became so, the more devastating were his comments about uncontrolled work. Those who attended Journal Club had another treat in store, for after the contents of current journals had been thoroughly discussed, Mrs. Rich, with all her graciousness and charm, joined the group for more general conversation. Then one obtained some insight into Dr. Rich's broad cultural interests, for in addition to medicine he touched on many other subjects: music, art, literature, and politics upon which his wide knowledge, sense of humor and his active imagination came into full play.

Unfortunately, only a few were privileged to collaborate with Dr. Rich in the laboratory. Those who did received a rare introduction to investigation and perceived at first hand his attention to detail, his clear ways of thinking and his high ideals.

Finally, past and present hospital associates will attest to the advice, criticism and inspiration they have derived from him with respect to their own problems, whether of a research or clinical nature. They found that any idea that survived his thorough examination deserved to live. Dr. Rich was the backbone of the Research Society, where informal appraisal of a member's work was beneficial for all. He was never too busy to look at a slide from a biopsy or an autopsy and discuss at length the implications with a surgeon or clinician, provided, of course, he was approached after twelve o'clock noon.

Such words as these inadequately express the stimulation and inspiration received by the student, the example left with a staff member, or the help given to an associate. Perhaps all has been summed up best by an elder statesman, "during the past four decades Dr. Rich has been the great critic of ideas, *the* intellectual center of this medical school".

I leave you to decide for yourselves whether our artist has caught his subject. Some of you had misgivings when you wrote, "I frankly cannot conceive of a painter being able to portray this man as I remember him in a state of repose. He moved so quickly, he thought so rapidly, he cerebrated so much more actively than those around him that a single picture could never relate the story". Or as another stated, "if the artist executing the work can capture that enigmatic smile, the *Mona Lisa* may have to move over".

I am sure this portrait will be a constant reminder, Dr. Rich, of the affection and gratitude which your classmates, students and associates have for you. And so, Dr. Turner, on behalf of Dr. Rich's Hopkins family, I take great pleasure in presenting this portrait to the Johns Hopkins University, whose Medical School and graduates have gained so much from the man whom we honor today.

THE ANTI-INFLAMMATORY EFFECT OF BUCCAL STREPTOKINASE-STREPTODORNASE[1]

A Controlled Double-Blind Study

PHILIP S. NORMAN and PAUL J. KADULL

The Department of Medicine, The Johns Hopkins University School of Medicine and Hospital and the U. S. Army Chemical Corps Laboratories, Fort Detrick, Frederick, Maryland

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Streptokinase and streptodornase were first introduced into therapy by Tillett and Sherry who described their combined use for the lysis of clotted or thick exudate in the pleural cavity (1). Shorty thereafter, these streptococcal enzymes were applied locally for "enzymatic debridement", i.e., the removal of necrotic tissue in surface wounds or ulcers (2). In both clinical applications a local concentration of the enzymes sufficient to achieve their characteristic activity is necessary for the desired therapeutic effect.

In 1955, Miller, Surmonte, Ginsberg, and Ablondi reported the intramuscular injection of streptokinase-streptodornase (SK-SD) for the nonspecific suppression of inflammation and edema in a number of local infections (3). Intramuscular trypsin (4) and chymotrypsin (5) have also been described as having anti-inflammatory effects in human disease. Absorption and distribution of these enzymes in an active form by the blood has never been demonstrated and it is unlikely with the rather small amounts injected that a concentration of enzyme adequate for proteolysis is achieved at sites distant from that of injection. Consequently, it has been suggested that intramuscular enzymes achieve their therapeutic effect through a generalized anti-inflammatory or "antiphlogistic" action occurring through an, as yet, unknown mechanism.

The anti-inflammatory effect of intramuscular SK-SD has found some confirmation in animal experimentation by Gordon and Ablondi who found that this agent reduced the inflammation due to instillation of mustard oil into rabbit eyes (6). Hamdy, Rheins, and Deatherage, however, were unable to show that SK-SD hastened the healing of bruises in rabbits unless injected directly into the bruise (7).

A recent development in systemic enzyme therapy has been the use of buccal tablets in order to save the patient from pain and discomfort at the intramuscular site of injection of enzymes. Miller *et al.* reported that SK-SD given buccally in conjunction with antibiotics brought about more rapid improvement in a variety of local infections and in thrombophlebitis (8). These authors did not attempt to assess how much of the enzyme was absorbed or what the duration of action was. Innerfield, Shub, and Boyd reported

[1] Supported by a contract with the U. S. Army Chemical Corps, Fort Detrick, Maryland.

similar clinical results in infection and thrombosis, and studied absorption of SK-SD in fourteen patients by the use of agar diffusion (9). After three weeks of treatment, they found an increase in the number or strength of the lines of precipitate formed by the patient's serum when tested against SK-SD. Whether these lines represented streptokinase or other materials in the SK-SD preparation was not determined, so their significance as to the absorption of active streptokinase remains in doubt. They also found that serum antithrombin titers were higher in patients treated with SK-SD than in normal individuals. However, serial determinations of serum antithrombin levels were made on these patients during treatment only, and the effect of the disease itself on these levels in the same patients was not assessed. Consequently, the best evidence for the absorption, distribution, and therapeutic effectiveness of buccal SK-SD was the clinical improvement in patients ill of a variety of inflammatory diseases, many of which improve even if untreated. Furthermore, most of these patients were being treated with antibiotics, anticoagulants, or both. No animal experimentation on the effect of buccal enzymes has been reported, probably because this method of administration is not practical in animals.

The experiments to be described here are an attempt to bring the antiphlogistic property of SK-SD into the realm of controllable and measurable phenomena. It is apparent from the above review that it is necessary to use the human subject with an inflammatory edematous lesion and that sufficient numbers of people are needed so that adequate control observations can be made. Such a lesion is found regularly at the U. S. Army Chemical Corps Laboratories at Fort Detrick, Maryland. A vaccine of alum-precipitated protective antigen from anthrax bacilli has been in use among personnel for a number of years (10). Booster injections are given every six months and sensitization to this vaccine is rather common as evidenced by an increasing rate of local inflammatory reactions with each succeeding booster injection. The principle manifestation is an edema which is inflammatory and non-pitting in character. The reaction may be pronounced and edema extending to the fingers has been seen from a deltoid inoculation. In some instances fever, malaise, or local adenopathy accompanies the more severe reactions. After a number of boosters, about 25 per cent of those receiving another injection may be expected to show a local reaction. The inflammation and edema accompanying these reactions represent the manifestations that are said to respond most dramatically to treatment with streptokinase. Consequently, a study was undertaken to determine whether SK-SD would suppress the development or hasten the resolution of these lesions.

PROCEDURE AND RESULTS

The double-blind technique was used and the patients received either drug or an indistinguishable placebo. Two hundred consecutively numbered packets

TABLE I

Criteria for Grading Local Allergic Reactions to Anthrax Vaccine

Trace	Erythema without edema
1+	Up to 5.0 cm. edema with erythema
2+	5.0 to 10.0 cm. edema with erythema
3+	10.0 to 15.0 cm. edema with erythema
4+	Over 15.0 cm. edema with erythema, central induration or enlargement of regional lymph nodes

TABLE II

Number of Persons with Local Reactions to Anthrax Vaccine

Grade of Reaction	SK-SD Treated*	Placebo Treated
4+	2	0
3+	4	9
2+	8	5
1+	8	6
Trace	11	11
Total Reactions	33	31
No Reaction	67	70
Total Treated	100	101

* Treatment with buccal SK-SD (Varidase) 40,000 units on the day of injection with the vaccine.

were prepared and were assigned either 40,000 units of Varidase[2] (four tablets) or placebo according to a table of random numbers. A packet was given to an individual when the regular booster inoculation was given and two tablets were taken at time of injection followed by two more eight hours later.

The patients returned at twenty-four and forty-eight hours, and the diameters in centimeters of the edema, erythema and induration were measured and recorded. The patients were questioned for systemic symptoms which might be the result either of the vaccine reaction or the drug. All reactions were subsiding by forty-eight hours so no further observations were made. Data for each patient were entered on individual cards. After all two hundred patients had been treated and their reactions recorded, the data cards were sorted into five groups according to the severity of the lesion. The criteria selected for grading the reactions from a trace to four plus are presented in Table I. The code was broken only after the lesions had been graded as to severity and then the results were evaluated as to the effectiveness of therapy.

The results of this trial are indicated in Table II and show that the number

[2] Varidase and Varidase placebos were kindly supplied by Dr. J. M. Ruegsegger, American Cyanamid Company, Pearl River, New York.

TABLE III

Number of Persons with Local Reactions to Anthrax Vaccine

Grade of Reaction	SK-SD Treated*	Placebo Treated
4+	0	0
3+	2	0
2+	2	6
1+	4	3
Total Reactions	8	9
No Reactions	38	35
Total Treated	46	44

* Treatment with buccal SK-SD (Varidase) 30,000 units for three days. Vaccine injection on the third day of treatment.

and severity of reactions were actually slightly greater in the treated group. If the trace reactions are counted as one-half, the two groups may be compared further by adding up the number of pluses in each column resulting in forty-nine and one-half pluses in the treated group and forty-eight and one-half in the placebo group.

This study might be criticized from the standpoint that results from buccal SK-SD usually take about twenty-four hours to become evident and that the full anti-inflammatory effect might not occur until several days of therapy have elapsed. Accordingly, a further series of subjects was studied after receiving SK-SD for three days. Thirty thousand units of Varidase or a placebo were administered daily for three days to one hundred persons. On the third day they were given their anthrax booster and the reactions followed as in the previous series with the same criteria for grading being used.

The results are shown in Table III. A different lot of vaccine was used to which the reaction incidence was 20 per cent, slightly less than the 25 per cent experienced with previous lots. Ten individuals did not take all their medicine and could not be included in the evaluations. Again the two most severe reactions were in the treated group; the pluses total fourteen in the treated column and fifteen in the placebo column. As these results were obtained with a somewhat smaller group, an attempt to assess their significance was made by calculating the 95 per cent confidence limits.[3] The 95 per cent confidence limits were −13 per cent to +19 per cent indicating that the result was compatible with a difference from that actually obtained which at the most was 19 per cent in favor of benefit from SK-SD and at the least was 13 per cent in favor of enhancement of inflammatory reactions. These limits include the

[3] The authors are grateful to Dr. Margaret Merrell for assistance in performing this calculation and in its interpretation.

TABLE IV

Side Effects Due to Buccal SK-SD (Varidase)

Side Effect	SK-SD	Placebo
40,000 Units in One Day (201 Subjects)		
Sore Throat	3	3
Headache	7	2
Stiffness	1	1
"Grippy"	1	0
Felt "Bad"	0	1
Chill	1	1
G.I. Upset	2	0
Ulcer on Gum	1	0
Dizzy	0	1
30,000 Units a Day for 3 Days (100 Subjects)		
Headache	2	1
Nausea	1	1
Ringing of Ears	1	0

value 0 indicating that the results could also be compatible with no effect at all.

A number of side effects were reported as indicated in Table IV, but it was apparent that these were almost as common in those receiving placebos as in those receiving the drug.

DISCUSSION

It would be unwarranted to conclude from this study that SK-SD given buccally has no anti-inflammatory effect in man. A single rather unusual lesion was studied only because it occurred in large enough numbers and in a setting where it could be subjected to a controlled study. If some ability to suppress these lessions had been seen, it would have been possible to undertake further studies to describe the dosage of the drug required for maximal effect, the duration of action and the development of tolerance, if any. These facts are lacking from current studies of the drug and, indeed, Innerfield, *et al.* have found dosages from 20,000 to 120,000 units daily to be equally effective and without serious side effects (9).

SK-SD has been offered as an adjunct to already established therapy in order to hasten improvement of a number of diseases that usually improve spontaneously. It seems evident, therefore, that widespread acceptance of this mode of therapy will only be obtained when controlled studies have demonstrated an anti-inflammatory effect in human disease in a situation where the bias of the investigator has been eliminated by the use of the double-blind technique.

SUMMARY

1. Using the double-blind technique, dosages by the buccal route of 40,000 units of SK-SD for one day or 30,000 units of SK-SD daily for three days were compared with placebos for their ability to suppress inflammatory allergic reactions in humans to an alum precipitated anthrax vaccine.

2. There was no significant difference in the numbers or severity of reactions between groups of individuals receiving SK-SD and those receiving placebos.

3. No significant side reactions to the drug occurred.

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CONNECTIVE TISSUE STUDIES[1]

II. The Effect of Vitamin C Deficiency on Healed Wounds

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In the chapter on the "History of Scurvy" in his monograph, *Scurvy, Past and Present*, Alfred Hess (1) presents an excellent summary. He notes that the ravages of scurvy have been recounted as historical occurrences since the campaigns of the Caesars, the pilgrimages of the Crusaders on land, and the voyages of the early explorers at sea.

In some of the early accounts, particularly that attributed to Richard Walter (2), who was chaplain to Lord Anson's expedition, the phenomenon is recorded which we have investigated and here present. Walter noted that the scars of wounds which have for many years healed were opened again when scurvy occurred. As an example, he describes an individual who had been initially wounded 50 years previously and whose wounds broke down and appeared as if they had never been healed when he contracted scurvy. A similar breakdown was described for a previously healed fracture. Lind (3) and Mead (4) are early writers who noted this breakdown of healed wounds following the occurrence of scurvy. Bourne (5) has reported the historical literature on scurvy and bone repair in his chapter on "Vitamin C and Bone". Recent reports on the effects of scurvy on healed wounds are sparse. Alan Hunt (6) in 1941 reported the effects of established scurvy on the healed wounds of three animals who had been deprived of all ascorbic acid from the twentieth day after operation. These studies were all histologic and showed changed staining qualities of the collagen of the scar. According to Hunt, "The ready reversion of the collagen of scars to its immature and weaker form offers an explanation of the breaking down of healed wounds in scurvy."

More recently, Pirani and Levenson (7) have made a histologic study of the effect of vitamin C deficiency in healed wounds. They performed midline laparotomy wounds and allowed them to heal for six weeks, during which period the animals were fed a nutritionally complete diet. They noted that after six weeks the wound scar was reduced to a thin line and was often hardly visible. In three instances where scurvy was produced, herniation developed

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[2] Resident House Officer Johns Hopkins Hospital 1923–24.

at the site of the wound; however, complete dehiscence did not occur. Histologic observations on the scar tissue of the scorbutic guinea pigs whose laparotomy wounds had healed normally six weeks prior to the initiation of the scorbutic diet revealed fibroblastic proliferation and regression of connective tissue elements and hemorrhages. In an editorial comment (8) on the experiments of Pirani and Levenson it was noted that adequate ascorbic acid intake is essential not only for normal healing in the period immediately following trauma but also for the maintenance of previously formed scar tissue. Scar tissue seems to be more susceptible to vitamin C deficiency than is normal tissue.

We have studied the effect of scurvy on two groups of animals; the first were animals with recently produced wounds, and we wish to compare the data obtained from them (9) with data obtained from a second group whose original wounds had been allowed to heal for long periods of time.

In the study here reported, Carbon^{14} data obtained from the use of L-ascorbic-1-C^{14} acid (10,11) are included in addition to the chemical determination of ascorbic acid. Measurements of the pressure of wound rupture following the withdrawal of ascorbic acid are correlated with the amounts of ascorbic acid determined chemically in the blood and tissues.

MATERIALS AND METHODS

Guinea pigs of the Hartley strain, 220 to 250 grams, were obtained from the same source of supply. For the operational procedure a midline abdominal incision was made as previously reported (9). The animals were maintained on a Reid-Briggs semi-synthetic diet (12) with the addition of 1 gm. of ascorbic acid to 1 kg. of feed mixture, which furnished each animal with a 30 mg. daily ascorbic acid intake.

The control animals were maintained on this diet postoperatively for 70 to 78 days. The animals in which scurvy was induced were on the 30 mg. daily ascorbic acid intake for 43 days after operation, following which time the vitamin was completely removed from their diet. They were maintained on the scurvy-producing diet from 28 to 35 days before sacrifice. The total length of the postoperative period of the control and scorbutic groups was the same. The technique to determine the strength of the wound by measurement of the pressure required for wound rupture was the same as described in a previous paper (9). Three animals in each group were given an intraperitoneal injection of 5 mg. of radioactive ascorbic acid three to seven hours before sacrifice. Samples of blood and adrenal glands were analyzed from each animal to ascertain the nutritional status. Specimens of skin and muscle were removed from the scar as well as from the abdominal wall 3 cm. distant from the scar for analysis at sacrifice as previously described (9). Chemical determination of ascorbic acid was made according to the method of Roe and Keuther (13). The ascorbic acid was prepared according to the method of Salomon, Burns, and King (11) and the cyanide was prepared according to that of von Schuching and Enns (10). The radioactivity was determined by measuring the rate of ion production with an electrometer (14). The analysis of radioactive carbon was made by preparing $BaCO_3$ by the wet combustion method (15). The $BaC^{14}O_3$ from each sample was brought to a total weight of 400 mg. with inactive $BaCO_3$ and sealed with 4 gm. of silver chloride-lead chloride *in vacuo* under anhydrous condition in a Pyrex

test tube, 19 by 200 mm. These test tubes were tapered as they were sealed. $C^{14}O_2$ was liberated by heating at 400° for four hours (16) in an aluminum block which held 20 sample tubes. After cooling, the samples were attached to the ionization chamber by way of the elongated neck of the sample container which was easily broken following evacuation of the system. For our ionization chamber a CO_2 sample pressure of 36 mm. indicated total liberation of CO_2 from 400 mg. of $BaCO_3$. The ionization chamber was brought to atmospheric pressure by slow introduction of air through a drying tower. The method of preparation insured uniformity of samples. The L-ascorbic-1-C^{14} acid after conversion to $BaCO_3$ was assayed against a Carbon^{14} standard in a methane preflush flow counter operated in the proportional counting region.[3] The activity was 2 μc. per mg. One mg. of the labeled ascorbic acid gave a reading of 5.4×10^3 divisions per minute for our ionization chamber after conversion to $BaC^{14}O_3$.[4]

RESULTS

In Table I we have compiled data which will be used as a basis for comparison with succeeding experiments. It should be noted that observations obtained from a single injection of L-ascorbic-1-C^{14} acid containing 5 to 10 mg. of inactive ascorbic acid as a carrier are difficult to interpret, particularly when administered to a scorbutic guinea pig, because the values are increased in the scar as well as in the distant tissues. The values for the ascorbic acid content of the scar tissue show a greater increase than the values for the distant tissue in all of the groups, regardless of dietary intake or mode of administration. The scar tissue contains considerably greater amounts of radioactive Carbon^{14} than does the distant tissue, which is a finding similar to that obtained by chemical determination.

In a study presented in Table II, a midline abdominal incision was performed on a group of animals and allowed to heal for 72 days. At sacrifice the wounds could not be ruptured. The dietary intake was 30 mg. daily.

A second group of animals whose wounds had similarly healed showed a weight gain equal to the control group up to the time they were placed on the ascorbic acid-free diet. Thereafter, until the time of sacrifice, these scorbutic animals lost considerable weight, while the control group continued to make a slight weight gain until the time of sacrifice.

The days between operation and sacrifice for the animals in which scurvy was produced averaged 76 days, while a similar period for the control animal group averaged 72 days. The average number of days for the animals on the scorbutic diet was 33 days. In previously reported experiments dealing with the younger growing animal, average survival was 21 to 24 days and our present findings are in agreement with Slack (17) who noted that older animals developed signs of scurvy more slowly and at more individual rates.

[3] RCL Nucleometer Preflush Flow Counter and Scaler.

[4] Carbon^{14} activity represents essentially all radioactive ascorbic acid 24 hr. after injection (Salomon, L. L., Dissertation, Columbia University, 1952: and our own data).

TABLE I

Data are presented for short-term (10-day postoperative) abdominal wound healing. Values for both chemical analyses and C^{14} radioactive ascorbic acid tracer studies on groups of animals on varying vitamin C intake are presented. The chemical and C^{14} radioactive determinations were made following single injections of radioactive ascorbic acid. The groups of animals were maintained on three different dietary intakes of ascorbic acid for comparison. The first three groups of determinations were made on scorbutic animals. The next three groups of animals received 6 mg. daily intake of ascorbic acid, which is a maintenance level; and the final three groups were on a 30 mg. daily intake of ascorbic acid, which is a saturation level. Chemical determinations of ascorbic acid are expressed in mg. ascorbic acid per 100 ml. or gm. and C^{14} values are expressed in per cent of dose of L-ascorbic-1-C^{14} acid injected per ml. or gm. tissue. Analyses were made on whole blood, adrenal glands, scar skin and muscle, and distant abdominal skin and muscle.

Mg. Ascorbic Acid Intake Per Day	No. of Animals	Days Between Operation and Sacrifice	Whole Blood	Adrenals	Scar Skin	Distant Abdominal Skin	Scar Muscle	Distant Abdominal Muscle
			mg. Ascorbic Acid per 100 ml. or gm.					
Chemical Determination								
0 mg.	5	10	.09 ± .01	6.5 ± .90	2.5 ± .30	2.4 ± .70	.8 ± .10	.9 ± .10
0 mg. intake + 10 mg. L-ascorbic-1-C^{14} acid injected intraperitoneally 24 hours before sacrifice	5	10	.16 ± .01	—	6.9 ± 1.00	2.6 ± .32	3.2 ± .25	1.2 ± .13
			C^{14} Values in Per Cent of Dose per ml. of gm. Tissue					
C^{14} Determination								
0 mg. intake + 10 mg. L-ascorbic-1-C^{14} acid injected intraperitoneally 24 hours before sacrifice	4	10	.08	—	.06	.11	.26	.23
			.09	—	.15	.09	.37	.08
			.04	—	—	—	.60	.15
			.06	—	.09	—	.15	.08
		Average	.07	—	.10	.10	.34	.14
			mg. Ascorbic Acid per 100 ml. or gm.					
Chemical Determination								
6 mg. mixed in food	5	10	.16 ± .04	32.7 ± 6.00	5.5 ± .90	4.1 ± .40	3.0 ± .50	1.7 ± .20
6 mg. intake + 10 mg. L-ascorbic-1-C^{14} acid injected intraperitoneally 24 hours before sacrifice	5	10	.36 ± .01	—	15.8 ± 2.42	8.0 ± .72	8.4 ± .92	3.7 ± .32

acid injected intraperitoneally 24 hours before sacrifice								
			C^{14} Values in Per Cent of Dose per ml. or gm. Tissue					
C^{14} Determination								
6 mg. intake + 10 mg. L-ascorbic-1-C^{14} acid injected intraperitoneally 24 hours before sacrifice	5	10	—	—	.14	.06	.11	.03
			.04	—	—	.05	.07	.02
			.03	—	—	.04	.10	.02
			.04	—	.14	.19	.14	.08
			—	—	.30	.26	.29	.07
		Average	.04	—	.19	.12	.14	.04
			mg. Ascorbic Acid per 100 ml. or gm.					
Chemical Determination								
30 mg. by dropper	5	10	.22 ± .02	45.0 ± 7.80	6.6 ± .32	4.0 ± .43	3.7 ± .29	1.7 ± .18
30 mg. intake + 10 mg. L-ascorbic-1-C^{14} acid injected intraperitoneally 24 hours before sacrifice	2	10	.47	197.00	11.10	3.20	10.50	4.20
			.45	108.00	—	—	11.70	8.70
		Average	.46	152.00	11.10	3.20	11.10	6.45
			C^{14} Values in Per Cent of Dose per ml. or gm. Tissue					
C^{14} Determination								
30 mg. intake + 10 mg. L-ascorbic-1-C^{14} acid injected intraperitoneally 24 hours before sacrifice	2	10	—	3.10	.26	.22	.19	.36
			.20	1.60	—	—	.32	.61
		Average	.20	2.35	.26	.22	.26	.48

TABLE II

Study of Abdominal Wounds in Guinea Pigs Which Had Been Allowed to Heal for a Long Period (Approximately 70 Days Postoperatively)

Comparison of Weight, Pressure of Wound Rupture and Ascorbic Acid Content in Blood and Tissues in a Scorbutic Group and in a Group on a 30 mg. Daily Ascorbic Acid Intake.

Animal No.	Guinea Pig Weights in gm.			Days Between Operation and Sacrifice	Days on 30 mg. Ascorbic Acid Intake Following Operation	Days on Scorbutic Diet Prior to Sacrifice	Pressure Wound Rupture mm. Hg		Milligrams per 100 ml. (Whole Blood)	At Sacrifice				
										Milligrams Ascorbic Acid per 100 gm. Tissue				
	At Operation	At Start of Scorbutic Diet	At Sacrifice				Skin	Muscle		Adrenals	Scar Skin	Distant Abdominal Skin	Scar Muscle	Distant Abdominal Muscle
1	275	419	339	71	43	28	300	300	.12	10.50	5.47	2.20	3.11	.93
2	333	462	329	71	43	28	370	370	.10	15.00	3.18	3.44	1.38	.53
3	306	430	346	77	43	34	370	370	.03	3.40	1.10	1.52	.52	.32
4	359	472	364	77	43	34	350	350	.04	4.10	2.53	1.18	.57	1.53
5	360	437	451	78	43	35	290	290	.05	3.90	1.37	.85	.63	.28
							—	—						
							336*	336*						
6	326	575	396	78	43	35	Could not rupture		.12	9.90	3.08	2.41	2.83	.87
7	361	534	464	78	43	35	Could not rupture		.11	8.00	2.32	2.28	1.82	.79
Average...	331	476	384	76	43	33	(See above)		.08	7.83	2.72	1.98	1.55	.75
1	362	438	409	78	78	0	490	Could not rupture	1.27	184.00	8.97	5.64	5.97	2.76
2	320	450	540	73	73	0	Could not rupture		.46	117.00	6.43	5.34	5.15	1.67
3	364	514	541	70	70	0	Could not rupture		.51	110.40	5.95	4.22	4.85	1.63
4	352	502	496	70	70	0	Could not rupture		.63	78.20	6.67	4.14	3.94	1.95
Average...	350	476	496	72	72	0	—	—	.72	122.00	7.00	4.84	4.98	2.00

* Average.

In the scorbutic group the pressure for wound rupture for both the skin and the muscle averaged 336 mm. of Hg for the five individual pigs in which rupture occurred. In two pigs in the scorbutic group neither rupture in the skin or muscle layer could be produced.

In the control group a rupture of the skin layer was produced in only one animal and this occurred at a high pressure of 490 mm. of Hg. In none of the control group of animals could a rupture of the abdominal muscle layer of the scar be affected.

In the scorbutic group, the ascorbic acid level in the whole blood ranged from .03 to .12 mg. per 100 ml., with an average of .08 mg. per 100 ml. In the control group, the values ranged from .46 to 1.27 mg. per 100 ml., with an average value of .72 mg. per 100 ml. The average ascorbic acid content of the adrenals in the scorbutic group was about $\frac{1}{15}$ of the average content of the control group. In the scorbutic group the ascorbic acid content of the scar skin was 33 per cent greater than the distant abdominal skin, while the ascorbic acid content of the scar muscle was 100 per cent greater than the distant abdominal muscle. These differences are accentuated in the control group on the 30 mg. daily intake of ascorbic acid. Here we find the ascorbic acid content of the scar skin approximately 50 per cent greater than the distant abdominal skin, and the scar muscle shows an increase of 150 per cent over the distant abdominal muscle.

This significant increase in the ascorbic acid content of the scar skin and

TABLE III

C^{14} Determinations Calculated as Per Cent of Administered Dose of L-Ascorbic-1-C^{14} Acid in Blood and Tissues in the Two Groups of Animals Whose Wounds had been Allowed to Heal for a Long Period (Approximately 70 Days Postoperatively)

Animal No.	5 mg. L-ascorbic-1-C^{14} Acid Injected Intramuscularly Before Sacrifice	Days Between Operation and Sacrifice	C^{14} Values in Per Cent of Dose per ml. or gm. Tissue					
			Whole Blood	Adrenals	Scar Skin	Distant Abdominal Skin	Scar Muscle	Distant Abdominal Muscle
Scorbutic								
1	5 hr.	76	.001	6.7	—	—	.64	.45
2	3 hr.	76	—	2.2	.12	.16	.60	.16
3	3 hr.	76	.07	4.2	.17	.15	—	.07
Average....			.03	4.4	.14	.16	.62	.23
Control								
1	3 hr.	72	.22	3.4	.33	.19	.22	.06
2	6½ hr.	72	—	3.5	—	.11	.50	.12
3	7 hr.	72	.04	3.0	.19	.16	.16	.08
Average....			.13	3.3	.26	.15	.29	.09

* Average.

TABLE IV

Ascorbic Acid Values of a Sample of Human Skin Obtained from a 30-Year-Old Lumbar Scar

Site of Scar	Duration of Scar	Ascorbic Acid, mg. per 100 gm.	
		Scar Skin	3 cm. Distant Abdominal Skin
Right lumbar area.....	30 yr.	9.0	5.5

scar muscle is similar to our results obtained in a group of younger animals in which only a 10 day interval existed between operation and sacrifice as previously reported (9).

Results of studies with L-ascorbic-1-C^{14} acid injected intramuscularly into the groups of animals whose wounds had been allowed to heal for a long period are noted in Table III. Here again the values for ascorbic acid in the scar tissue are higher than in the distant tissue.

Human Skin Analysis. In a sample of human skin from a 30-year-old lumbar scar the ascorbic acid content of the scar tissue was 64 per cent greater than a sample of normal skin tissue 3 cm. distant. The patient was on a high vitamin C intake before the specimens were removed.

DISCUSSION

We have confirmed experimentally in the guinea pig that wounds healed for a long period will break down in scurvy. This finding substantiates the observation of the physicians who accompanied the early explorations, as well as findings based on histologic studies on animals (6, 7).

From the fact that the previously healed wound in scurvy ruptures, we must conclude that ascorbic acid is essential for the integrity of scar tissue. In contrast, Elster (18) admits that ascorbic acid is essential for the formation of collagen, but notes that it may not be essential for the maintenance of preformed connective tissue.

We have noted that in scurvy the chemical values of ascorbic acid are at practically the same level in the scar as in the distant tissue for recently healed wounds in which all tissues are depleted in scurvy. A single injection of L-ascorbic-1-C^{14} acid to the scorbutic guinea pig brings out immediately the increased deposit of ascorbic acid in scar tissue.

We had previously noted the increased accumulation of ascorbic acid in recent scar tissue at various levels of vitamin C dietary intake. We wished to ascertain whether this increased accumulation would decline to the values found in an unoperated animal after a period of time had elapsed. Our results indicate that the higher level of ascorbic acid in the scar tissue persists both in the guinea pig and in man. We have demonstrated by the injection of L-

ascorbic-1-C^{14} acid that it contributed to the increased ascorbic acid level in the scar tissue, regardless of the age of the postoperative scar.

CONCLUSIONS

1) Abdominal wounds in the guinea pig which had been allowed to heal for a long period could be ruptured in animals after scurvy had been produced. Similar wounds could not be ruptured in animals on adequate vitamin C intake.

2) Adequate ascorbic acid intake is essential, not only for immediate postoperative healing, but also for the maintenance of previously formed scar tissue.

3) Ascorbic acid accumulates in scar tissue immediately following wounding and persists for long periods of time both in the guinea pig and in man.

4) The higher levels of ascorbic acid noted in scar tissue are derived in part from the recently administered ascorbic acid as demonstrated by single injections of radioactive ascorbic acid.

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ELECTROPHORESIS OF HEMOGLOBIN ON AGAR GELS

Frequency of Hemoglobin D in a Negro Population[1]

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The use of agar gels as supporting media for the electrophoresis of proteins was described by Gordon and his associates (1, 2) and by Bussard and Perrin (3). Giri (4, 5, 6, 7, 8) and subsequently other investigators (9, 10, 11, 12) successfully employed an agar medium for the electrophoretic separation of hemoglobins. Robinson and his associates (9) demonstrated two special virtues of the agar gel technique: 1. fetal and normal hemoglobins can be widely separated, and 2. hemoglobin D is clearly distinguished electrophoretically from hemoglobin S. The specific usefulness of the method for definitive diagnosis of sickle cell-hemoglobin D disease has been recognized (12).

In the investigation to be described a method of electrophoresis on agar gels has been improved so that reproducible and permanent preparations of high quality can be simply prepared employing techniques suitable for large surveys. Conditions influencing the rate of migration of hemoglobin on agar have been examined. The method has been applied to a variety of clinical problems and has been employed in a survey to determine the frequency of occurrence of hemoglobin D in American negroes.

TECHNIQUE OF ELECTROPHORESIS

The electrophoresis apparatus was slightly modified from that of Smith and Conley (13). Marginal acrylic plastic strips, 1/16 inch by 1/4 inch, were glued to the surface of a sheet of plate glass measuring 10 by 12 by 1/4 inches, producing a shallow tray 1/16 inch in depth. The glass plate was preheated in an oven at 110°C to prevent premature gelling of the agar. A solution containing 2 grams agar[3] in 200 ml. of citrate buffer was heated until clear and poured on the plate, completely filling the volume enclosed by the plastic strips. By use of a straight edge the fluid agar was cleared of bubbles and planed to the level of the 1/16 inch plastic strips. Before gelation occurred filter paper wicks approximately 10 by 6 inches were inserted at the end of the plate under the surface of the agar. These wicks had previously been molded over the edges of the dry glass plate so that they would fit snugly over the rim, with 1½ inches of the filter paper completely immersed in the agar. After gelation was complete, strips of filter paper 1/4 by 1/8 inch were placed 1/4 inch apart *on the surface* of the gel along the midline of the plate. Approximately 6 cubic millimeters of hemolysate were applied by pipette to each strip of filter paper.

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[3] "BACTO-AGAR" Difco Laboratories.

After the hemoglobin solutions were applied, the entire surface of the gel was covered with a quick-drying clear liquid plastic spray[4] to prevent drying of the agar film during electrophoresis. Care was taken not to disperse the hemolysates nor to impregnate the filter paper wicks. When the liquid plastic layer had dried, the plate was placed on the lips of the buffer tanks with the wicks dipping into the buffer. Approximately 200 volts was applied across the plate for 8 hours at room temperature (about 24°C).

After electrophoresis the wicks were removed and the plastic sheet carefully stripped from the agar film. Excess agar at the ends of the plate was cut free and discarded and the glass plate placed in a shallow pan. The plate was covered with bromphenol blue stain (prepared by dissolving 100 mg. of the dye in 1000 ml. distilled water and adding 10 ml. glacial acetic acid). The agar film was manipulated free of the glass plate, and a thin sheet of wax[5] was slipped under the agar. The wax sheet with intact agar superimposed was removed from the pan and drained of excess stain. The edges of the wax sheet were secured by glass plates to prevent wrinkling while the agar dried. The completely dried agar united with the wax base to form a paper-thin durable sheet. The composite sheet was mounted on filter paper with plastic adhesive tape. The mounted preparations were filed as permanent original records.

Hemoglobin solutions were prepared from blood to which an anticoagulant had been added. Approximately 2 ml. of packed erythrocytes were washed twice with 0.9 per cent sodium chloride solution. Four ml. distilled water and 0.5 ml. toluol were added to the packed red cells and the mixture was agitated in a mechanical shaker for 10 minutes to produce hemolysis. After centrifugation at a relative centrifugal force of 2000x gravity for 20 minutes, the stromal residue and toluol were removed with suction. The final concentration of hemoglobin was approximately 8 to 10 gm. per 100 ml.

Sodium citrate-citric acid buffer, 0.05M, pH 6.0, ionic strength approximately 0.05, was prepared by dissolving 147 gm. sodium citrate in 600 ml. distilled water, adjusting the pH to 6.0 with 50 per cent citric acid solution, and diluting to 1000 ml. with distilled water. This stock solution was diluted with 9 volumes of distilled water and the pH adjusted to 6.0 with citric acid before use. Citrate buffer of other pH values was prepared in similar fashion.

The method of Smith and Conley (13) was employed for electrophoresis of hemoglobin on filter paper.

RESULTS

1. Electrophoretic patterns

When hemoglobin from normal blood was subjected to electrophoresis on agar plates in citrate buffer at pH 6.0, migration was toward the cathode. Three distinct components became apparent (Fig. 1). The major component was hemoglobin A. Two minor components moved more rapidly and constituted small fractions of the total. That these minor components consisted of hemoglobin was shown by their intense staining with the benzidine reagent.

The faster moving minor component of normal hemoglobin had the same mobility as the major component of hemoglobin obtained from umbilical cord blood and is therefore presumed to be fetal (F) hemoglobin. Electrophoresis of hemoglobin from newborn infants and from patients with thalassemia

[4] "KRYLON" No. 1301 Spray coating.
[5] "Parafilm" grade "M", Marathon.

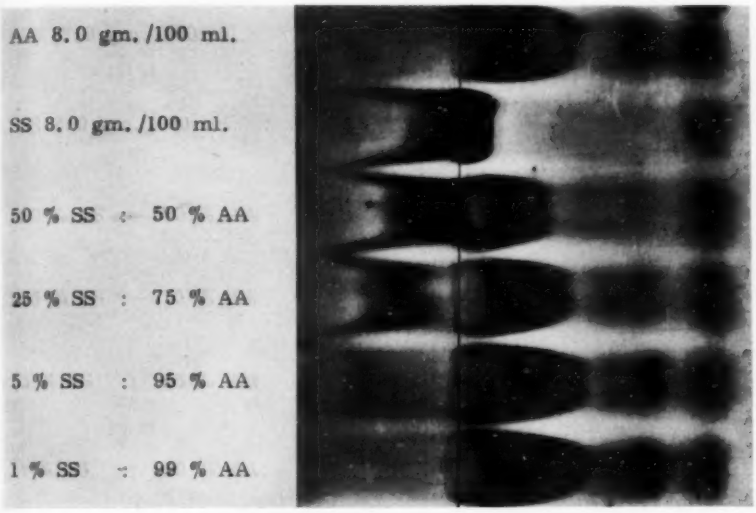

FIG. 1. Patterns of hemoglobin from a normal adult and from a patient with sickle cell anemia are shown on agar gel after 8 hours of electrophoresis in citrate buffer at pH 6.0. The starting position is indicated by the vertical line. The three components of normal hemoglobin (AA) are clearly seen. A spot with the mobility of fetal hemoglobin is present in the SS pattern, which, however, lacks the second fast-moving component. In mixtures of normal and sickle cell hemoglobin, each with a total hemoglobin concentration of 8.0 gm. per 100 ml., the intensity of the second minor component parallels the concentration of hemoglobin A. The rate of migration of hemoglobin A is directly related to its concentration. The same effect is seen with hemoglobin S, which in low concentration actually moved toward the anode, probably as a result of electroosmotic flow. To be noted are the characteristic differences in the appearance of the spots: the minor components are rounded, hemoglobin A is bullet shaped, and hemoglobin S is more streaked.

major, sickle cell-thalassemia disease, sickle cell anemia and other disorders in which the concentration of fetal hemoglobin was increased, showed a more intense spot in this position (Fig. 2).

The second fast-moving minor component of normal hemoglobin has not been identified. The intensity of this spot was directly related to the concentration of hemoglobin A in the hemolysate (Fig. 1). This component was not seen in the patterns prepared from blood which did not contain hemoglobin A, including blood from patients with sickle cell anemia, sickle cell-hemoglobin C disease, homozygous hemoglobin C disease and sickle cell-hemoglobin D disease. It was also not detected when the relative concentration of hemoglobin A was low, as in cord blood and in blood from some patients with sickle cell-thalassemia disease. The possibility was considered that this minor component might be hemoglobin A_2, the slow moving fraction observed on

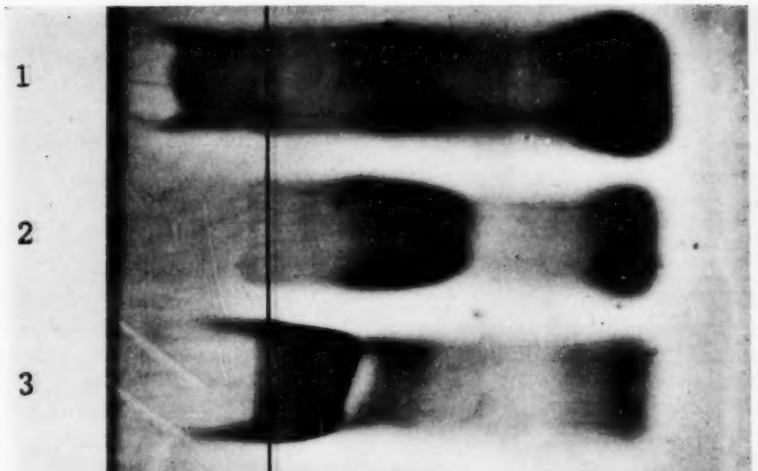

FIG. 2. Variations in the concentration of fetal hemoglobin are shown in these electrophoretic patterns on agar gel (pH 6.0). The rate of migration of hemoglobin F was not significantly influenced by changes in concentration. Hemolysates were prepared from blood obtained from: (1) umbilical cord of an infant with sickle cell trait. The S and A components are visible but the major fraction is hemoglobin F. (2) a normal infant of 2 months showing hemoglobins A and F. (3) an adult with sickle cell anemia. Hemoglobin S is in higher concentration here than in pattern No. 1 and accordingly has moved more in the direction of the cathode. (4) a normal adult. In this pattern the major component is hemoglobin A. A spot is present in the F position, but the second minor component cannot be detected. (5) an adult with sickle cell-thalassemia disease. The relatively high concentrations of hemoglobins S and F are apparent, but hemoglobin A and the second minor component are also present. (6) an adult with inherited persistence of fetal hemoglobin, showing predominantly hemoglobins A and F.

starch-block electrophoresis of normal hemoglobin. A concentrate of purified hemoglobin A_2 was accordingly studied.[6] However, when subjected to electrophoresis on agar, hemoglobin A_2 had the same mobility as hemoglobin A.

Hemoglobin S moved more slowly than hemoglobin A, and hemoglobin C more slowly than hemoglobin S. Hemoglobin from patients with sickle cell anemia and sickle cell-hemoglobin C disease often showed a small component which migrated in the A position and was clearly distinct from the S and fast-moving spots. Several hemoglobins which had mobilities faster than that of hemoglobin A on filter paper at pH 8.6 were subjected to electrophoresis on agar. These included Hopkins I and Hopkins II (14), two other genetically determined abnormal hemoglobins encountered in this clinic (15), and three fast-moving hemoglobins kindly supplied by Dr. Amoz Chernoff (hemoglobins I, J and Chernoff N). In addition the fast moving component encountered in this laboratory in 9 of 20 patients with lead poisoning was studied. None of these abnormal components was detected on the agar plates and all apparently had the mobility of hemoglobin A by which they were obscured.

For the first two hours of electrophoresis, all of the hemoglobin components appeared as rounded spots. Fetal hemoglobin retained this form. By the end of 8 hours, the A, S, C, and D spots had bullet-shaped configurations, with side trails streaking toward the anode. The C spot at times remained at the origin and often moved slightly toward the anode. There were variations in the positions of the A, S, C, and D spots, depending mainly on the concentration of these hemoglobins in the hemolysates. This phenomenon is described below and illustrated in Figure 1.

Hemoglobin D had the same mobility on agar as hemoglobin A. Individuals heterozygous for hemoglobins A and D had only a single spot in the A position. When hemoglobin from such persons was subjected to electrophoresis on filter paper in barbital buffer at pH 8.6, the pattern was indistinguishable from that of sickle cell trait. Therefore, a hemoglobin sample which showed an SA pattern on paper but an AA pattern on agar was identified as DA rather than SA (Fig. 3). On filter paper the electrophoretic pattern of hemoglobin from a patient with sickle cell-hemoglobin D disease could not be differentiated from that of sickle cell anemia. However, on agar the SD hemolysate showed major components in the S and A positions, whereas the SS hemolysate showed only a single major component in the S position.

2. Conditions influencing the migration of hemoglobin

A. pH. As the pH of the buffer was reduced, there was a characteristic alteration in the appearance of the hemoglobin spots (Fig. 4). Fetal hemoglobin

[6] Purified concentrate of hemoglobin A_2, eluted from starch block, was prepared and generously provided by A. O. W. Stretton, Massachusetts Institute of Technology, through the courtesy of Dr. Vernon Ingram.

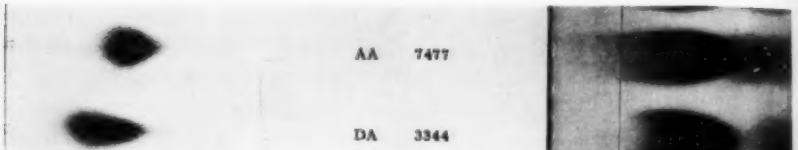

FIG. 3. Electrophoretic patterns on filter paper (veronal buffer, pH 8.6), left, are compared with patterns of the same hemolysates on agar (citrate buffer, pH 6.0), right. Hemoglobins S and D are not separated on filter paper but are clearly distinguished on agar. By simultaneous use of both techniques, SA patterns are readily differentiated from DA, and also SD from SS patterns.

FIG. 4. Effects of changes in pH of the citrate buffer on electrophoretic mobility of hemoglobins on agar are shown. Hemoglobins A and F have the same mobility until pH is reduced to a level at which the A spot becomes bullet-shaped. The sequential development of changes in the contour of the various hemoglobin spots with decreasing pH is demonstrated. The long side-trails of hemoglobin C are clearly seen.

persisted as a rounded density until the pH was reduced below 5.8. At pH 5.4 this hemoglobin had assumed a slightly bullet-shaped pattern. In contrast, hemoglobin C had a strikingly swept-back appearance even at pH 6.8. At the latter pH, hemoglobins A and D appeared as rounded spots and hemoglobin S was somewhat bullet-shaped. The different contours of these hemoglobin spots permitted their recognition with reasonable accuracy even apart from their relative positions. When the pH was less than 5.8, the A, S, C and D spots appeared as dense bullet-shaped patterns, all with the same mobility. Only the fast-moving F component of cord blood and the side trails of hemoglobin C could be distinguished at these low pH values. Best resolution of

the major and minor hemoglobin components was observed at pH 6.0. Acceptable though less well defined patterns were obtained in the pH range 5.8 to 6.2. Hemoglobin D had the same mobility as hemoglobin A throughout the pH range tested (5.4 to 6.8).

When citrate buffer at pH 6.0 was used for paper electrophoresis, migration was toward the cathode. All hemoglobin patterns were rounded and diffuse and not bullet-shaped. Hemoglobin S migrated slightly faster than hemoglobin A, but resolution of the hemoglobin components was inadequate for accurate identification. Under these conditions, hemoglobin D had the same mobility as hemoglobin S.

When barbital buffer at pH 8.6 was employed in the agar preparation, hemoglobins A, S, C, D and F appeared as rounded spots with the same relative rates of migration as on filter paper at this pH. Agar gels in barbital buffer were unsuitable for permanent preparations, because of crystallization of the buffer after drying.

The pH of the citrate buffer was measured after electrophoresis in agar. Although the pH changed considerably in the electrode compartments (from pH 6.0 to 5.0 in the anode tank; from pH 6.0 to 11.8 in the cathode tank), the buffer in contact with the filter paper wicks was not measurably altered after 8 hours.

B. SIZE OF THE AGAR FILM. The width of the agar film was a matter of no apparent importance in influencing the migration of hemoglobins. By using glass plates twelve inches in width, fifteen or more samples of hemoglobin were subjected to electrophoresis simultaneously. The thickness of the agar film, however, was of critical significance. Best results were obtained when the liquid agar was planed to the level of the $\frac{1}{16}$ inch rim of plastic stripping. Thicker agar films resulted in less migration of the spots toward the cathode and less sharply defined components, even when the voltage was increased. With films $\frac{1}{4}$ inch thick, only slight migration occurred, and there was no separation of the hemoglobin components. The technique employed had the advantage of yielding films of uniform and reproducible thickness, with consequently uniform rates of migration.

C. HEMOGLOBIN CONCENTRATION. When various concentrations of the same hemoglobin specimens were run simultaneously on agar, it was apparent that the rate of migration was a function of the hemoglobin concentration. Spots containing higher concentrations of hemoglobins A, S and C moved more rapidly than spots containing lower concentrations of the same hemoglobin (Fig. 1). This effect was much more pronounced in electrophoresis on agar films than on filter paper. The use of hemoglobin solutions of equal concentrations was therefore necessary for comparative studies, since a large amount of hemoglobin C migrated at about the same rate as a small amount of hemoglobin S. Confusion was avoided by assessing the density and contour of the

spots as well as their relative positions. The volume of hemoglobin solution used also influenced the migration of the spots. A greater volume of hemolysate migrated farther than a lesser volume of the same hemolysate. The migration of fetal hemoglobin changed only slightly with variations in concentration and volume (Fig. 2).

3. Sensitivity of the agar gel technique

A component in the position of fetal hemoglobin was seen in every electrophoretic pattern. This spot was large and intense in hemoglobin obtained from cord blood, and was faint and barely visible in hemoglobin from many normal adults. Mixtures of hemoglobin S and hemoglobin A, both in concentrations of 8 grams per 100 ml., were run on agar to demonstrate the smallest amount of hemoglobin S which could be detected. As shown in Figure 1, hemoglobin S was barely visible in a mixture containing 5 per cent hemoglobin S, and undetectable in a concentration of 1 per cent. The cord blood of an infant with sickle cell trait (SA) showed S and A spots which could be readily recognized, together with a dense spot in the fetal position (Fig. 2). When the hemoglobin of infants was tested on agar gel electrophoresis at monthly intervals, one could follow the increase in hemoglobins S and A, coincident with the decrease in fetal hemoglobin content.

The agar method was employed to study hemoglobin from a family with inherited persistence of fetal hemoglobin (15, 16). In members of the family who had inherited this abnormal gene the agar pattern showed an intense spot in the position of fetal hemoglobin (Fig. 2). When this gene was present together with the gene for hemoglobin S, only hemoglobins S and F were detected on the agar films. In artificial mixtures, hemoglobin A was readily detected on agar plates when it comprised 3 per cent of the hemoglobin in the hemolysate. Therefore, those members of this family with the SF pattern must have had less than 3 per cent hemoglobin A, and possibly none at all.

4. Incidence of hemoglobin D in a Negro population

Since hemoglobin D has the mobility of hemoglobin S on filter paper in barbital buffer at pH 8.6, and the mobility of hemoglobin A on agar in citrate buffer at pH 6.0, simultaneous use of these techniques simplified the recognition of hemoglobin D. Blood specimens were submitted from Negroes seen in various clinics of The Johns Hopkins Hospital as part of a large scale screening survey to determine the incidence of abnormal hemoglobins. When hemoglobin components from these individuals had the mobility of hemoglobin S on filter paper, the red blood cells were tested for the sickling phenomenon and an aliquot of the hemoglobin specimen was subjected to electrophoresis on agar.

During the course of this survey, 400 persons were found to have an SA pattern on filter paper. Four of these individuals were shown to have hemoglobin D rather than hemoglobin S, and their red cells could not be made to sickle using sodium metabisulfite. Blood was obtained from 91 individuals in whom a diagnosis of sickle cell anemia had previously been made, in an effort to determine whether some of these patients had hemoglobin D, and all were found to have only hemoglobin S as the major component. Electrophoresis on agar of hemoglobin from 20 patients with sickle cell-hemoglobin C disease showed only hemoglobins S and C as major components.

DISCUSSION

Electrophoresis of hemoglobin on agar plates by the method described is a simple procedure suitable for large surveys and yielding clear and reproducible results. An important advantage of this method is that the original patterns form permanent records which can easily be filed for future reference.

The configuration and rate of migration of the hemoglobin spots on agar gels in citrate buffer are probably dependent on a number of factors, including 1) the net charge of the hemoglobin molecule, 2) alterations in hemoglobin produced by the low pH of the buffer, 3) adsorption of the hemoglobin components onto the agar medium, and 4) electroösmotic flow.

Fetal hemoglobin appears to be resistant to change as the pH is reduced, since deviation from the rounded pattern did not occur until the pH was below 5.8. Interestingly, the rate of migration of fetal hemoglobin was not significantly influenced by large changes in hemoglobin concentration. Hemoglobins A, S, C and D were apparently altered at these low pH values, and must have undergone changes which account for their peculiar contours. The rate of migration of these hemoglobins was markedly influenced by changes in concentration. Hemoglobins S and D were separable only when agar was contained in the medium, suggesting the possibility of differential adsorption of these hemoglobins onto the agar gel framework.

Of the two minor fast moving components of normal hemoglobin, one has the mobility of fetal hemoglobin. It is not certain, however, that all of the hemoglobin with this mobility is in fact fetal in type. The proportion of normal hemoglobin which this fraction represents is considerably in excess of the concentration of fetal hemoglobin as determined immunochemically or by the alkali denaturation technique. Further studies will be necessary to determine the composition of this fast moving component. Nevertheless, the agar technique is extremely useful in the investigation of conditions in which fetal hemoglobin is increased in concentration. The method is much more satisfactory than filter paper electrophoresis for measuring progressive changes in the distribution of hemoglobins during the newborn period and in early life. In the study of a family with hereditary persistence of fetal hemoglobin,

results with the agar technique have led to the reasonable hypothesis that the abnormal gene suppresses the formation of hemoglobin A (15).

The second fast-moving component has not been identified with a known fraction of normal hemoglobin, although its presence was clearly associated with the presence of hemoglobin A. The appearance of this component must be influenced by minor variations in the technique since it was not invariably demonstrated. Hemoglobin A_2 as well as all of the hemoglobins tested, which on filter paper have rates of migration faster than hemoglobin A, were not separable from hemoglobin A on agar.

Four of 400 Negroes who had SA patterns on filter paper electrophoresis were shown to have hemoglobin D trait by agar gel electrophoresis and by the absence of the sickling phenomenon. Since the frequency of the sickle cell trait in the Negro population of Baltimore is about 85 per 1,000 (13), one can estimate the incidence of hemoglobin D trait in this population to be 0.85 per 1,000. A previous survey (12) of 1,000 Negroes in Baltimore revealed that one person had hemoglobin D trait, a result identical with that obtained in a similar survey in New York (17). Chernoff (18) reported that 4 of 1,000 Negroes in St. Louis were found to have hemoglobin D trait, an incidence much higher than in the present study.

On the basis of the surveys carried out in this laboratory, approximately 0.18 per cent of Negroes at birth can be expected to have sickle cell anemia and 0.0018 per cent to have sickle cell-hemoglobin D disease. Because of the high mortality associated with these diseases, their incidence in the total Negro population is undoubtedly much lower. It is not surprising, in the study of 91 cases of presumed sickle cell anemia, that no case of sickle cell-hemoglobin D disease was discovered. It is noteworthy, however, that all of the reported cases of S-D disease have occurred in non-Negroes (12, 19, 20, 21), an observation suggesting that instances of the disorder in Negroes have been overlooked. The clinical manifestations of sickle cell anemia and sickle cell-hemoglobin D disease are similar. Furthermore, sickling of the red cells occurs prominently in both disorders and the electrophoretic pattern of hemoglobin on filter paper in the two diseases is identical. Therefore, it seems likely that cases of S-D disease in Negroes have been mistakenly identified as sickle cell anemia. A continuation of the present study is certain eventually to detect cases of previously unrecognized S-D disease. The number of occasions on which S-D disease has been discovered in white persons, particularly in view of the rarity of hemoglobin S in Caucasians, indicates that hemoglobin D is probably more common in white populations than in Negroes.

SUMMARY

1) A simple method has been devised for electrophoresis of hemoglobins on agar gels. Reproducible patterns of high quality are obtained and the original patterns form durable records.

2) Factors influencing the migration of hemoglobin on agar have been studied. Of considerable practical importance is the demonstration that with this method hemoglobin concentration importantly influences rate of migration.

3) The unique property of agar to permit separation of hemoglobins A and F, and hemoglobins S and D probably depends on differential alterations in these hemoglobin molecules at low pH, and differing degrees of adsorption of the altered hemoglobins to the agar medium.

4) The agar gel technique was employed in the study of a number of clinical problems and was utilized in a survey to determine the frequency of hemoglobin D in Negroes in Baltimore.

5) Of 400 Negroes who had sickle cell trait patterns on filter paper electrophoresis, 4 were shown actually to have hemoglobin D trait (AD), indicating a frequency of D trait of about 0.085 per cent in the Negro population of Baltimore.

6) Ninety-one Negroes previously considered to have sickle cell anemia were restudied to determine if some might in fact have sickle cell-hemoglobin D disease. In every instance the electrophoretic patterns of hemoglobin were those of homozygous sickle cell disease (SS) and no new case of SD disease was discovered.

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ABSTRACTS OF PAPERS PRESENTED BEFORE THE MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY IN ASSOCIATION WITH THE ALPHA OMEGA ALPHA FRATERNITY

May 18, 1959

THE EFFECT OF INTESTINAL MOTILITY ON ADHESION FORMATION

DONALD F. CONE

Paper published in the July 1959 issue of the Bulletin of the Johns Hopkins Hospital

THE RENAL CLEARANCE OF CITRIC ACID IN THE DOG

ARTHUR P. GROLLMAN

The effects of acidosis, alkalosis, fluoroacetate, Diamox, and certain dicarboxylic acids of the Krebs cycle on the renal excretion of citric acid in the dog have been investigated by means of standard clearance techniques. The exogenous creatinine clearance was used as a measure of the glomerular filtration rate; citrate and substrates studied were infused intravenously, and metabolic alkalosis and acidosis were effected by oral sodium bicarbonate or ammonium chloride administration.

Citrate clearances were markedly increased by alkalosis and diminished by acidosis. Diamox decreased the citrate clearance even after systemic alkalosis was induced suggesting that Diamox has an effect on the reabsorption of citrate unrelated to its influence on systemic and urinary pH. This effect was overcome by increasing the concentrations of citrate in the plasma to greater than normal values.

At elevated plasma citrate concentrations the citrate clearances were less than the glomerular filtration rate both during metabolic alkalosis and after fluoroacetate poisoning. The citrate clearances become equal to the creatinine clearances following infusion of sodium malate at normal plasma bicarbonate concentrations and could not be further elevated by superimposed alkalosis. Sodium succinate had an identical effect, one which was not influenced by addition of the succinic dehydrogenase inhibitor, malonic acid. During these experiments the concentration of citrate in the plasma remained at normal levels. In the malate experiments an increase of the plasma citrate by intravenous infusion of citrate reduced the citrate to creatinine clearance ratio from 1 to values of 0.8 or 0.9. Utilization of citrate, as calculated from renal arteriovenous differences and simultaneous urinary excretion measurements, was strikingly inhibited by the malate infusion.

These data are consistent with a renal mechanism of citrate excretion involving filtration and reabsorption but not secretion. The reabsorption and subsequent utilization of citrate by the tubule cell appear to be competitively inhibited by the Krebs cycle substrates, malate and succinate.

EXCHANGE OF LABELED METHIONINE BETWEEN HEMOGRAFT AND HOST

HARVEY G. KEMP, JR.

Paper to be published in its entirety

SUCCINIC DEHYDROGENASE AND ITS INHIBITION BY FUMARATE AND THYROXINE

NORMAN L. LASSER

Paper to be published in its entirety

ELECTROPHORESIS OF HEMOGLOBIN IN AGAR GELS

VICTOR J. MARDER

Paper to be published this issue of Bulletin

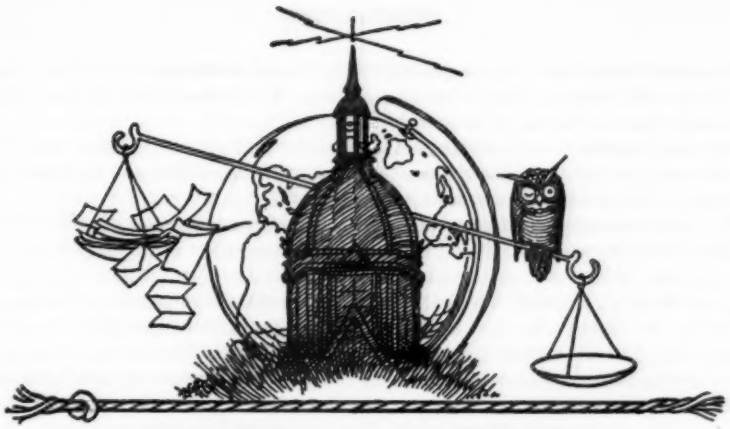
CENTRAL INHIBITION OF AUDITORY STIMULI

ROBERT J. RUBEN AND JAN SEKULA

Electrical stimulation of an area about 2 mm. in diameter and 10 mm. rostral to the obex, on the dorsal surface of the brain stem of the cat, inhibits the response of the 8th nerve to a click, as has previously been reported. This area is that of the decussation of the olivo-cochlear bundles of Rasmussen, which constitute an efferent innervation to the organ of Corti.

Using voltages of stimulation lower than needed to inhibit the 8th nerve response to a click, the evoked potential at the auditory cortex was suppressed. In some animals the cortical potential to a click stimulus was totally abolished without any change in the 8th nerve response. The responses at the inferior colliculus and the medial geniculate were suppressed with higher voltages than needed for cortical suppression but lower than needed to abolish the 8th nerve responses.

Section of the olivo-cochlear bundle just lateral to the decussation caused the suppression phenomena to cease. Use of neuromuscular blocking agents had no effect.



BOOK REVIEWS

Therapeutic Electricity and Ultraviolet Radiation. Vol. IV of Physical Medicine Library. Edited by SIDNEY LICHT. 373 pp., $10.00. Elizabeth Licht, Publisher, New Haven, Conn.

For some time there has been an obvious need for an organized, comprehensive and critical presentation of the field of Physical Medicine. Previous volumes in this series have covered the fields of electrodiagnosis, therapeutic heat and therapeutic exercise. The present volume covers the history, instrumentation, physiological effects and clinical use of electricity, iontophoresis and ultraviolet radiation. The historical section by Sidney Licht is especially interesting. One is amazed at the amount of time and energy which has been expended in the application of electricity to the treatment of patients over the last 200 years and how little of practical value has survived. The sections on instrumentation are models of clarity and comprehensiveness.

Of particular interest is Keith Stillwell's careful summary of the physiologic, histological, chemical and electromyographic changes which occur with denervation of a muscle, and the experimental and clinical evaluation of the effects of therapeutic electrical stimulation on the prevention of muscle atrophy during nerve regeneration. Ernst Fischer and Sidney Solomon have an excellent chapter on the physiologic effects of ultraviolet radiation. The book is well indexed with excellent bibliographies following each chapter.

Unfortunately, the field of Physical Medicine, while precise and meticulous in the measurement of the modality (heat, electricity, ultraviolet light, and to a lesser extent exercise) applied to the patient, must depend largely on the subjective reaction of the patient to judge therapeutic success. There are few reports of matched series, double blind tests or other methods of evaluation of results which might eliminate the subjective reactions of patient and doctor. Nor is it easy to design such experiments, when the patient can tell whether he is or is not being treated with a potentially therapeutic modality. Nevertheless, until such experiments can be designed, therapy with physical modalities will continue to depend on clinical impressions.

This most recent volume summarizes past experience and present indications for therapeutic electricity and ultraviolet radiation. It should remain the definitive exposition of these modalities for some time to come.

DOUGLAS CARROLL

Treatment of Cancer and Allied Diseases. Vol. 3, Tumors of the Head and Neck. Edited by GEORGE T. PACK AND IRVING M. ARIEL. 781 pp., $30.00. Paul B. Hoeber, Inc., Medical Book Dept. of Harper & Bros., New York, N. Y.

This book represents an impressive addition to the field of head and neck surgery. The volume contains seven-hundred-eighty-one pages, consisting of fifty-one separate chapters written by seventy authors selected by the editors.

The book is organized into several sections. The first, "General Principals," includes sections on anesthesia for head and neck surgery, preoperative and postoperative care in the treatment of head and neck neoplasms and a description of the method of the spread of cancer in the head and neck. The second section deals with tumors of the oral cavity, pharynx, nose, and sinuses. The third and fourth sections deal with tumors of the ear and eye, respectively. The fifth section deals with tumors of the larynx. The sixth section deals with tumors arising in the neck primarily, including tumors of the carotid body, techniques of neck dissection and a chapter on radiotherapy of carcinoma, metastatic to cervical lymph nodes. The final section deals with tumors of the thyroid gland and parathyroid glands.

The book is nicely printed on glossy paper with one-thousand-twenty eight illustrations of excellent quality of reproduction. The authors state in their preface, "because of the protean problems of cancer of the head and neck, no *one* method of management can be declared 'the best.' In this book, the editors have presented all therapeutic methods, whether radical or conservative, that are at present acceptable for tumors of the head and neck (exclusive of brain tumors)."

Separate chapters are devoted to surgical and radiologic treatment of given cancers with indications and contraindications, techniques, present accomplishments and complications so that the reader may select the method of his choice on the basis of information presented.

The importance of this work can be judged by the fact that 14.7 per cent of all deaths from cancer are due to cancer of the head and neck. In the head and neck, the crowding of tissues is so marked that vital structures are early invaded by tumor. The surgeon and the therapist are certainly not independent units in handling malignant tumors of the head and neck. The section on anesthesia of the head and neck, deals with many of the special problems concerned with having an anesthetist and a surgical team working in the same small anatomical region. This reviewer was somewhat disappointed that they did not suggest the technique of introducing the tracheotomy, under local, (whenever it is to be used) as the initial procedure of anesthesia. In this way, the patient's larynx is saved the trauma of intubation. Techniques of deep cervical block and superficial cervical block are nicely illustrated.

The section on cancer of the lip points out the interesting fact that lower lip cancer is twenty times as common as that of the upper lip and that this condition is singularly rare in the negro. Males are effected in 95 per cent of the cases.

One of the possible objections that might be leveled at this interesting book is that in an effort to present all of the various thoughts in the care of head and neck cancer, the reader has been left with little editorial guide as to the *limitations* of the various methods and might indeed have some difficulty in selecting the most desirable method from the standpoint of cure in any given tumor. For that reason, the book will probably be of greatest usefulness to men who have had already considerable experience in the management of this disease.

Throughout the book numerous helpful tables on survival, mortality, and recurrence rate are given with various methods of therapy. Unfortunately, the reader is unable to tell in many instances, whether the cases reported represent "total experience" of the authors with that type of disease, or whether in fact they represent only "those cases treated by that

method." However, the general level of statistical reporting is better here than in most previous volumes on cancer results. The book is quite readable and contains many up-to-date references pertinent to the subjects. The editors have added at the end of many of the chapters notes to include more recent material than was available to the author of that chapter at the time of writing. This is an important feature in making the material up-to-date. All in all, this book will prove useful to those treating patients with tumors of the head and neck and will offer much valuable basic information to students in the field.

MILTON T. EDGERTON

Textbook of Microbiology, 17th edition. By WILLIAM BURROWS. 954 pp., $14.00. W. B. Saunders Co., Philadelphia, Pa.

The compleat microbiologist, like the compleat man, is a disappearing breed. Today, the mycologist finds closer kinship with the dermatologist, the parasitologist with the epidemiologist, the bacterial virologist with the biophysicist, and the microbial geneticist with the protein chemist than with other microbiologists. Therefore, it would be quite a *tour de force* for a single person to write authoritatively about the entire field of microbiology. The author of this textbook has succeeded to a remarkable degree, assisted only in bacterial metabolism (by Dr. R. J. Porter) and in medical parasitology (by Dr. J. W. Moulder). In the first chapters of the book, which deal with principles and methods, accurate information on the biology of bacteria and viruses is well synthesized and correlated. Inescapably, the latter half degenerates into a telegraphic account of microbial species and genera, accompanied by the usual superficial descriptions of the diseases caused by each. References to the literature are far too few but appear to be relatively well chosen. Obviously, this volume is directed toward that large and amorphous market composed of sophomore medical students. The reviewer has no fixed opinions about the aspects of microbiology to which they should be exposed. However, it is not likely that this textbook will capture the imagination of the truly interested neophyte.

ROBERT R. WAGNER

Pediatric Neurology. By STANLEY S. LAMM. 495 pp., $12.90. Landsberger Medical Books, New York, N. Y.

This is said, in the brochure, to be designed as a practical book on pediatric neurology. It is certainly written by a pediatrician with especial interests in that field, and has chapter headings covering a wide range. No doubt, viewed from that angle, it may serve a useful purpose in directing the attention of pediatricians and medical students to the increasingly prominent neurological problems of childhood. It cannot, however, be considered, from a neurological standpoint, a satisfactory presentation of those problems, or even their frankly "practical" aspects. Descriptions are sketchy and uncritical in many instances, and anatomical, physiological or pathological bases of correlation insufficient.

J. W. MAGLADERY

A History of Neurology. By WALTHER RIESE. Foreword by Felix Marti-Ibanez. 223 pp., $4.00. MD Publications, New York, N. Y.

This book is not a chronological history of neurology, but a history of what the author considers to be the dominant ideas of the structure and function of the central nervous system. Because the ancients ultimately settled on the cranial cavity as the seat of the soul and because this belief has persisted in one form or another to modern times, the history of neurology has been linked to metaphysics for more than 2,000 years. By tracing back through history ideas in modern neurology the author reveals our indebtedness to the intellectual

giants from Plato to Hughlings Jackson and expresses the belief that insofar as the neurological sciences ignore those historical precedents they are in peril of losing their foundation in logic.

There are short chapters devoted to the history of the nervous impulse, reflex action, pain, diagnosis, prognosis and therapy in neurology, but more than half the book is devoted to the doctrine of cerebral localization and the relation of brain structure to brain function. It is evident that the author prefers the "holistic" view of the function of the nervous system, in fact of the entire organism, to that of localization of function. Evidence advanced to support the view of the brain as a functional whole centers about recovery of function following brain injury *e.g.*, recovery from hemiplegia following large destructive lesions of "motor areas" and the failure of efforts to assign aphasic syndromes to specific anatomical areas in the brain. Nevertheless, he acknowledges the value of regional diagnosis of central nervous system lesions, thus vitiating his argument somewhat. It appears that in apposing the concept of the brain as a functioning whole to the doctrine of cerebral localization he does not adequately distinguish between cerebral localization of the most elementary property of vision, for example, and localization of memory or mind. With regard to the latter and insofar as the rapid advances in the neurological sciences in the past 20 years have led to renewed speculation on the anatomical and physiological substrates of behavior the author's admonitions are timely.

This is a difficult but erudite book which should be rewarding to the reader interested in the history of the mind-brain problem and many of its metaphysical connotations.

THOMAS W. LANGFITT

Basic Issues in Psychiatry. By PAUL V. LEMKAU. 106 pp., $3.50. Charles C Thomas, Springfield, Ill.

Dr. Lemkau's unique presentation is a small book (one hundred and six pages) with big dimensions. The style is palatable and warm; as though one is listening to a series of interesting lectures. Yet, the material is presented concisely and clearly in a fashion that is easily retained.

Any text-book in a specialized field often has a limited range of appeal. "Basic Issues in Psychiatry" can be a stimulating if not necessary part of the library for the medical profession—from the medical student to the well-learned psychoanalyst. Perhaps the only complaint about the book is the selection of the title, since it does not convey the appeal and breadth contained within its covers. Non-medical readers should find considerable information about a most pressing problem in our culture today.

The approach to the understanding of mental illness is stimulating and accurate. When Dr. Lemkau states, "Just what the devil is psychiatric disease?" one is immediately prepared for a new kind of thinking. For example, considering mental disease a number of illnesses rather than one single unit makes the reader recognize the vastness of the problem. Statistics, impressions, and validity are all conveyed in the style described above; as for example, "All statistics on mental illness thus far have showed that it is a good idea to be married."

For the psychiatrist who has become overly sophisticated, sections of the book will remind him of forgotten illnesses that are still necessary to be taken into account. Furthermore, explanations of confusing terminology in the field of psychiatry are made with ease; for example, the concept of "secondary gain."

What is usually missing in most psychiatric text-books is the understanding of how mental illness may be prevented. One finds in Dr. Lemkau's text practical issues presented that have far-reaching implications. The chapter on the prevention of psychogenic illness is excellent. The final chapter on the administration of psychiatric program can be described only by the word provocative. Not only must one think differently about how psychiatric

illness is treated and accepted, but one is forced into healthy speculation about the kind of world in which we live. The bibliography in Dr. Lemkau's book is worthy of mention for those interested in reference material.

Thus, whether one views the book, "Basic Issues in Psychiatry," as a text for medical students, a handy reminder to the trained psychiatrist, a tool to add to the knowledge of the physician in general, or an exposé to the public, one must consider this book one of the best things that has come upon the scene of the over-abundant medical literature that is available today.

LEONARD J. GALLANT

Cardiac Arrest and Resuscitation. By HUGH E. STEPHENSON. 378 pp., $12.00. The C. V. Mosby Co., St. Louis, Mo.

This book is a detailed review of all clinical cases of cardiac arrest reported in the literature. Over 1500 references are listed in the bibliography. The historical summary of the development of methods of cardiac resuscitation is complete and excellent.

The book might have been more effectively written if compressed to about 100 pages. Much of the bulk of the text consists of quotations from numerous surgical articles, many of which are opinions, and do not represent any factual data. The book could have been condensed by limiting the material included to definitive clinical and experimental data and excluding the reports that were not possible to analyze and consisted mostly of opinions. The book is valuable, however, as a complete reference source of all clinical reports of cardiac arrest.

The author quite properly emphasizes the importance of speed in treatment, initiating cardiac resuscitation within four minutes after arrest has occurred. A second point well emphasized is the importance of effective massage, as massage by inexperienced individuals is often ineffective. His emphasis on mouth-to-mouth ventilation is good; almost no other method is available within the time limits required for successful cardiac resuscitation. His emphasis on aortic or intracardiac transfusions is not supported by experimental evidence; rapid infusions into large veins are equally effective and are less hazardous. It is interesting that the reflex origin of cardiac arrest is considered more important than anoxia; considerable evidence is listed to support this viewpoint.

The book is an excellent reference source for all clinical experiences with cardiac arrest. It is too long to be a good book for medical students. The printing, binding, and illustrations are well done.

FRANK C. SPENCER

Anatomy for Surgeons. Vol. 3, The Back and Limbs. By W. HENRY HOLLINSHEAD. 901 pp., $23.50. Paul B. Hoeber, Inc., Medical Book Dept. of Harper & Bros., New York, N. Y.

This volume, the last of a three volume set covering human anatomy, considers the back and limbs in the same excellent style noted in volumes one and two. A general discussion of the development and structure of these regions is included as well as specific anatomical discussions. The orientation, organization, presentation and illustration of material is equally as attractive and readable as in the previous volumes. Emphasis is again placed on the anatomical aspects, but the correlation of this material with embryologic, physiologic and clinical considerations makes the presentation particularly effective.

Enthusiastic approval should be given this volume and its companions. They represent an excellent addition to the literature of anatomy and will be of particular value to surgeons, regardless of their stage of training. They may be heartily recommended to student and surgeon alike.

JAMES R. CANTRELL

BOOKS RECEIVED FOR REVIEW

"Allergic" Encephalomyelitis. Proceedings of a Symposium on Experimental "Allergic" Encephalomyelitis and Its Relation to Other Diseases of Man and Animals. Edited by MARIAN W. KIES AND ELLSWORTH C. ALVORD, JR. 576 pp., $13.50. Charles C Thomas, Publisher, Springfield, Ill.

Anatomy of the Human Body. By R. D. LOCKHART, G. F. HAMILTON AND F. W. FYFE. 697 pp., $13.50. J. B. Lippincott Co., Philadelphia, Pa.

Annual Review of Medicine, Vol. 10, 1959. Edited by DAVID A. RYTAND. 448 pp., $7.00. Annual Reviews, Palo Alto, Cal.

Blood Groups. British Medical Bulletin, Vol. 15, No. 2, May 1959. 174 pp., $3.25. Published by the Medical Department, The British Council, London, England.

The Functions of the Endocrine Glands. By PETER F. HALL. 290 pp., $5.75. W. B. Saunders Co., Philadelphia, Pa.

Hypertension Edited by JOHN H. MOYER. 790 pp., $14.00. W. B. Saunders Co., Philadelphia, Pa.

Medical Science and Space Travel. By WILLIAM A. KINNEY with an introduction by MAJ. GEN. HARRY G. ARMSTRONG, USAF (Medical Corps). 150 pp., $3.95. Franklin Watts, New York, N. Y.

Orthopaedics. Principles and Their Application. By SAMUEL L. TUREK. 906 pp., $22.50. J. B. Lippincott Co., Philadelphia, Pa.

Pathologic Physiology of Oral Disease. By RICHARD W. TIECKE, ORION H. STUTEVILLE AND JOSEPH C. CALANDRA. 480 pp., $11.50. The C. V. Mosby Co., St. Louis, Mo.

Patient Care and Special Procedures in Xray Technology. By CAROL HOCKING VENNES AND JOHN C. WATSON. 203 pp., $5.75. The C. V. Mosby Co., St. Louis, Mo.

Physiology of Cardiac Surgery. Hypothermia, Extracorporeal Circulation and Extracorporeal Cooling. By FRANK GOLLAN. 96 pp., $4.50. Charles C Thomas, Publisher, Springfield, Ill.

A Textbook of Medicine, Vols. I and II, 10th edition. Edited by RUSSELL L. CECIL AND ROBERT F. LOEB. 1754 pp., $20.50. W. B. Saunders Co., Philadelphia, Pa.

Your Mind Can Make You Sick or Well. By CURT S. WACHTEL. 244 pp., $4.95. Prentice-Hall, Inc., New York, N. Y.

ENLARGEMENT AND ROUND CELL INFILTRATION OF THE SALIVARY GLANDS ASSOCIATED WITH SYSTEMIC DISEASE

PALMER H. FUTCHER

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Enlargement of the salivary glands, and the parotid glands in particular, is an event which is both impressive to most patients and is easily recognized by an examining physician. Further, such enlargement is sometimes associated with serious systemic disease. These considerations prompted the ensuing analysis of a group of subjects suffering from illnesses of obscure etiology, and often of obscure identity, with which enlargement of the salivary glands was associated.

The salivary glands become enlarged as a result of a variety of processes. Included are such relatively benign illnesses as epidemic parotitis and obstruction of the ducts due to salivary calculi. Acute pyogenic infections of the gland sometimes occur as complications of the dehydration encountered in serious illness. Sarcoidosis can involve these glands, as, of course, can neoplasms. Enlargement of the gland has been described in patients with diabetes mellitus (1, 2, 3), liver disease (4, 5), and chronic malnutrition (3, 6, 7, 8, 9); in the last instance the enlargement is apparently due to a hypertrophy of the acinar tissue.

It is the purpose of this paper to report ten patients observed at The Johns Hopkins Hospital in whom there occurred a chronic or recurrent enlargement of a salivary gland which could be ascribed to none of the causes just mentioned; the subjects were demonstrated to manifest, or were strongly suspected of manifesting, a serious systemic disease. A salivary gland showed lymphocytic infiltration and decrease in the acini in the eight patients in whom microscopic examination was made.

Others have already pointed out the association of parotid abnormalities with disease of obscure etiology elsewhere in the body (10). Sjögren (11) emphasized the association of rheumatoid arthritis with disease of the parotid and lacrimal glands. Evidence supporting the general concept that the histologically very similar parotid derangement in Sjögren's and Mikulicz's syndromes is related to a systemic disease has been marshalled by Morgan (12). Parotid enlargement has been described in Felty's syndrome (13, 14). Enlargement of the parotid gland has been reported to occur in lupus erythematosus (15, 16, 17, 18), as has xerostomia without recognized enlargement (19). Swelling of the parotid has been reported as occurring in

TABLE I

Clinical and Laboratory Data on Selected Subjects with Enlargement of Salivary Glands

Subject	Age 1st Seen	Age Last Seen	Sex and Race	Salivary Gland Abnormalities	Ophthalmic Abnormalities	Other Abnormalities	Laboratory Studies						Summary
							Age	L.E. Cell	Sed. Rate	Serum Globulin	Hypergammaglobulinemia	Biopsy	
	yrs.	*yrs.*					*yrs.*		*mm. per hr.*	*gm. %*			
R. A. 699565	55	57	F W	Bilateral parotid swelling (55).	None.	Stiffness fingers, knees, jaws, pigmentation, scaly rash, weakness (54). Perioral constriction, lymphadenopathy (55). Fusiform fingers (56).	55 56	neg. neg.	29 25	4.8 5.8	 pos.	*Muscle* (55): Arteritis. *Parotid* (56): Lymphoid infiltration. *Skin of finger* (56): Scleroderma.	Salivary and systemic manifestations,* probable scleroderma. Died (56).
L. B. 728209	58	58	F W	Bilateral parotid swelling (52). Xerostomia (53).	Irritation (54). Schirmer test abnormal (58).	Arthralgia (48). Pigmentation lower legs, possibly longstanding and related to varices (58).	58	neg.	32	3.2	pos.	*Parotid* (58): Spotty lymphocytic infiltration, calculus in small duct.	Salivary, lacrimal, and possible systemic manifestations.
M. C. 740674	56	56	F W	Xerostomia (55). Right parotid nodule (56).	Itching, redness (55). Schirmer test abnormal (56).	Onset rheumatoid arthritis (44). Progressive deformities hands and feet.	56	neg.	34	4.3	pos.	*Parotid* (56): Lymphoid infiltration, with germinal centers.	Salivary and lacrimal manifestations; rheumatoid arthritis (Sjogren's syndrome).
M. J. 609604	57	64	F W	Bilateral parotid swelling, xeros-	Photophobia, lacrimation, Schir-	Erythematous macular eruption (60). Pain	57 60 62	 neg.	22 28 26	2.3 2.1 2.2	n.e.	*Skin* (60): Lupus erythematosus. *Skin* (62): Pos-	Salivary, possible lacrimal, and systemic manifesta-

				ing, xeros-	tion, Schir-	tion (60). Pain	62	neg.	26	2.2		*Skin* (62): Pos-	temic manifesta-

				tomia, dental caries (52).	mer test normal (60).	fingers, wrists, elbows, purpuric eruption, slight fever, following tetanus antitoxin (61).	64		20	2.6	neg.	sible collagen swelling. *Muscle* (62): Normal.	tions. Possible lupus erythematosus.
H. P. 411439	34	38	F W	Bilateral parotid swelling (32).	None.	Arthritis ankles, knees, weight loss (32). Lymphadenopathy, splenomegaly, fusiform fingers (34).	34	n.e.	40	3.2	n.e.	*Parotid* (34): Lymphoid infiltration with germinal centers, fibrosis, epithelial islands. *Nodes* (34): Hyperplasia. *Muscle* (34): Focal perivascular infiltration.	Salivary and systemic manifestations; possible rheumatoid arthritis.
E. R. 160326	41	42	F W	Bilateral parotid swelling (37).	Conjunctivitis, burning (35). Photophobia, palpebral conjunctivitis (41).	"Rheumatic fever" (26, 38). Pleurisy (30). Pericarditis (34, 38). Anosmia, loss of taste (38). Vomiting (40).	41 42	n.e.	30 31	n.e.	n.e.	n.e.	Salivary, possible lacrimal, and systemic manifestations.
L. R. 724736	43	45	F W	Bilateral parotid swelling, xerostomia (40).	Complaint "irritation" (41). Schirmer test abnormal (43).	Non-thrombocytopenic purpura, splenomegaly (43).	43	neg.	31	4.3	pos.	*Parotid* (43): Lymphoid infiltration, epithelial islands. *Node* (43): Inflammation.	Salivary and systemic manifestations.

TABLE I—*Continued*

Subject	Age 1st Seen	Age Last S	Sex and Race	Salivary Gland Abnormalities	Ophthalmic Abnormalities	Other Abnormalities	Laboratory Studies						Summary
							Age	L.E. Cell	Sed. Rate	Serum Globulin	Hypergammaglobulinemia	Biopsy	
	yrs.	*yrs.*					*yrs.*		*mm. per hr.*	*gm.%*			
J. S. 827648	59	59	M C	Bilateral parotid swelling (57). Bilateral submaxillary swelling (58).	Palpable lacrimal glands (59).	Epistaxes (56). Fatigue (58). Left abdominal and neck discomfort, marked splenomegaly, skin ulcer, leg edema (59).	59	neg.	12†	4.9	†	*Submaxillary* (59): Lymphoid infiltration, epithelial islands. *Muscle* (59): Slight atrophy. *Skin* (59): Normal.	Salivary, possible lacrimal and systemic manifestations, with abnormal serum protein and anemia.†
L. S. 629267	45	45	F W	Bilateral parotid swelling (45).	Reddening, sticking (45).	Arthralgia, fever, weight loss (43). Lymphadenopathy, pneumonitis, splenomegaly, hepatomegaly, abdominal pain (45).	45	pos.	36	3.8	n.e.	*Parotid* (45): Lymphoid infiltration. *Node* (45): Follicular hyperplasia.	Salivary, lacrimal, and systemic manifestations; probable lupus erythematosus. Died (45), no autopsy.
R. S. 659771	31	36	M W	Bilateral parotid swelling (29).	Complaint "irritation" (22).	Fever, arthralgia, pneumonia (22). Pneumonia (28). Arthralgia persisting (36).	31	n.e.	39	3.0	n.e.	*Parotid* (29, 32): Lymphoid infiltration, epithelial islands.	Salivary and systemic manifestations.

"n.e." signifies "not examined".

Figures in parentheses indicate age of patient.

* Patient R. A.: Hematocrit 28 vol. per cent.

† Patient J. S.: Serum cryoglobulin, cardiolipin test for syphilis, Reiter treponeme compliment fixation, cold hemagglutinins—all positive. Abnormal globulin at point of application on electrophoresis paper. Albuminuria. Fecal urobilinogen elevated. Hematocrit 18 vol. per cent. Uncorrected sedimentation rate 73 mm. per hr. Reticulocytes 9 per cent. Leucocytosis (14,700), with 64 per cent lymphocytes.

polyarteritis (17, 20, 21) and acroscleroderma (17). It has also been observed in patients with purpura not associated with leukemia (22, 23). "Sialadenitis" has been reported to be among the previous illnesses of apparently healthy subjects manifesting persistent unexplained elevation of the sedimentation rate; in these subjects there was demonstrated an increase in the concentration of serum gamma globulin (24). Elevation of the serum globulins also occurred in patients already referred to above (15, 22) as manifesting parotid enlargement. Xerostomia, suggesting disease of the parotids, has occurred in association with idiopathic cryoglobulinemia (25) and with disorders in which hyperglobulinemia was observed (26).

METHOD OF STUDY

Presented in tabular form are the abnormalities observed in ten patients in whom swelling of salivary glands was associated with obscure systemic disease. During a review of the case records of The Johns Hopkins Hospital there were encountered numerous such instances. From the group were selected patients on whom enough clinical and laboratory information had been compiled to warrant presentation (Table I).

The rotated buffy coat technique of Zinkham and Conley (27) was employed in the identification of L.E. cells in the blood with the exception of the examination of the blood of Subject L. S. In the latter instance the preparation was made in another hospital, and the presence of L.E. cells was confirmed by the division of hematology at the Johns Hopkins Hospital. Erythrocyte sedimentation rate was measured in a Wintrobe tube, and the values were corrected for abnormalities in the hematocrit (28); rates in excess of 20 mm. per hour were regarded as abnormal. Serum globulin was measured by a micro-Kjeldahl method after employing the Kingsley (29) technique of globulin precipitation; normal concentrations in the Hospital laboratory are rarely in excess of 3.5 grams per 100 ml. Serum gamma globulin was roughly quantitated by paper electrophoresis; in all subjects indicated in Table I as manifesting hypergammaglobulinemia, the concentration of gamma globulin in the serum was estimated to be 200 per cent of normal, or greater.

CLINICAL ANALYSIS

The majority of the ten patients we describe in Table I were women over 40 years of age. Of the salivary glands, the parotids were those most noticeably involved by swelling. In nine instances the swelling was bilateral. Xerostomia was sometimes noted. One subject, M. C., had not herself noted the abnormality in her salivary gland—a unilateral parotid nodule. The enlargement in most subjects persisted for several years, and was painless much of the time. In several subjects there were occasional variations in the promi-

at point of application on electrophoresis paper. Albuminuria. Fecal urobilinogen elevated. Hematocrit 18 vol. per cent. Uncorrected sedimentation rate 73 mm. per hr. Reticulocytes 9 per cent. Leucocytosis (14,700), with 64 per cent lymphocytes.

nence of the swelling. Most of the patients complained of ocular symptoms suggesting a conjunctival disorder. In only one subject, J. S., was lacrimal gland enlargement noted, and in that instance the increase in size was minimal.

Most patients complained of some joint symptoms, but in only one was impressive rheumatoid arthritis noted. Other manifestations presenting in two or more patients were lymphadenopathy, splenomegaly, and purpura.

Hyperglobulinemia was present in five of the nine subjects tested, and paper electrophoresis revealed an elevation of the serum gamma globulins in four of six subjects. In the serum of Subject J. S. there was present an abnormal serum globulin on electrophoresis, and a cryoglobulin and cold hemagglutinins were demonstrated. Elevation of the erythrocyte sedimentation rate was the rule. In Subjects M. C., M. J., H. P., E. R., L. R., and L. S. the white blood count was recorded on at least one occasion at 4,500 per cu. mm. or below.

The evidence is strong that Subject L. S. suffered from systemic lupus erythematosus; M. J., E. R., L. R., and R. S. may also have had that disease. Subject M. C. had rheumatoid arthritis with Sjögren's syndrome, and it is possible that L. B. had the same illness with only minimal joint disease. Whether H. P. suffered from rheumatoid arthritis, Felty's syndrome, or lupus erythematosus is uncertain. The illness of Subject R. A. suggested scleroderma. The cause of the very striking findings in J. S. remained uncertain. While there was considerable divergency in the manifestations of disease in these patients, it is possible that all of them suffered from one or another of the illnesses in the group of disorders of collagen tissue.

PATHOLOGIC FINDINGS IN SALIVARY GLANDS

The salivary glands of the eight subjects in whom biopsy was performed all showed impressive lymphoid infiltration; in two, germinal centers were prominent. The lymphoid tissue appeared to replace the acini, in some subjects to the extent that a few ducts only remained as vestige of glandular tissue. The sections in four of the subjects showed the myoepithelial islands emphasized as characteristic of Mikulicz's disease by Morgan and Castleman (30). (See Fig. 1.)

The specimens of salivary gland tissue available for pathologic study were usually quite small, sometimes less than one centimeter in diameter. It was therefore impossible to determine whether the tissue examined was representative of the whole gland. There was some variation from subject to subject in the microscopic findings, both as to the diffuseness of the lymphoid infiltration and the density of the infiltration in an involved area. In no instance was there any evidence of sarcoid.

The limited size of the biopsy specimens and the fact that material from

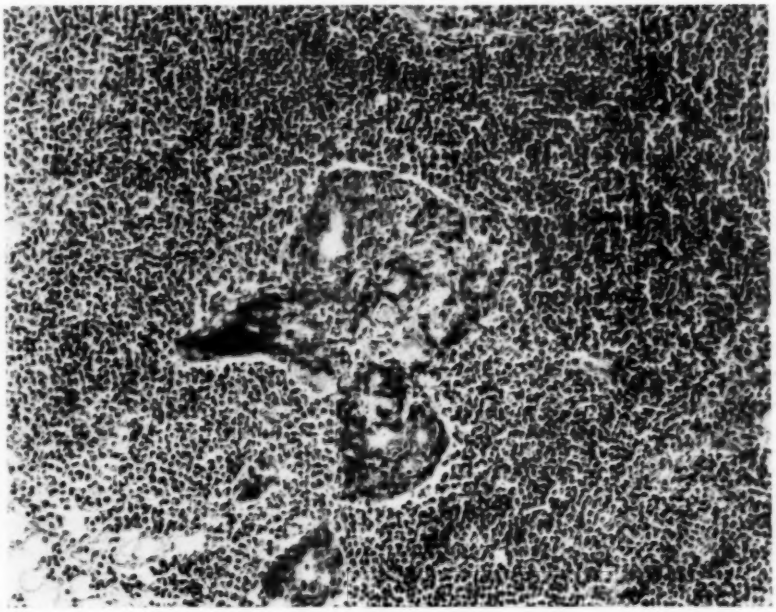

FIG. 1. Photomicrograph of parotid gland of Subject L. R. Lymphocytes have replaced the acini. A "myoepithelial island" occupies the center of the field. Magnification 200 ×.

only eight subjects was available preclude any meaningful correlation of the changes in the individual salivary gland with such factors as clinical diagnosis or the presence of hyperglobulinemia. The examination of the biopsy material from the eight subjects which has been conducted to date does not permit recognition of dissimilarity in the pathologic findings from subject to subject sufficient to demonstrate an important difference within the group. Myoepithelial islands were not recognized in the material from all eight subjects, and in two subjects lymphoid follicles were more prominent than was the case in the majority of the patients with Mikulicz's disease reported upon by Morgan and Castleman (30). However, in general the pathologic findings in the salivary glands seem very similar to those encountered by those authors and by Sjögren (11).

In our review of the histories of a group of patients not considered in Table I, we encountered some subjects in whom a parotid gland was enlarged by a lymphocytic infiltrate but in whom the salivary lesion appeared to be the only manifestation of disease. It is uncertain whether the cause of the salivary disease in these subjects was completely different from the cause or causes operating in the subjects listed in Table I, or whether there was failure to

detect existing more diffuse disease in the body. The usually unilateral nature of these uncomplicated cases suggests that the etiology may have differed from that operating in the subjects described in Table I, who usually manifested bilateral salivary gland disease.

DISCUSSION

In our patients with salivary gland swellings, it is uncertain why the lymphoid infiltration and disappearance of acini occurred. An association of lymphoid tissue with the structure of apparently normal salivary glands has been described, but is impressive only in infants (31, 32). Sjögren, writing of the sicca syndrome, pointed out the similarity between the pathologic findings seen in the lacrimal and those seen in the salivary glands (11); he believed that the lymphoid accumulation in these glands was secondary to a degenerative change in the acinar epithelium (33). Patey and Thackray (34) have directed attention to changes in salivary ducts in chronic parotitis, suggesting that epithelial proliferation may cause occlusion, with resulting acinar atrophy and lymphoid infiltration.

Local infection has been suggested as a cause of salivary disease similar to that seen in our subjects (31). Some support for this theory is lent by the fact that in several patients whose records we have reviewed there was rapid and marked variation from time to time in the degree of the swelling of the salivary gland. Sometimes a previously slightly enlarged parotid gland which had not been causing symptoms would become markedly swollen and painful, and fever would be recorded; the presence of an intercurrent acute bacterial infection seemed likely. That infections are actually a *primary* factor in the chronic salivary disease under discussion is, however, questioned by Rose (35), who is of the opinion that many patients in whom recurrent pyogenic parotitis is the most prominent symptom owe their salivary infections to the depressed salivary flow occasioned by Sjögren's disease. Patey and Thackray (34) believe that their studies support the hypothesis that the pathologic process in the parotid gland is the same in patients with chronic parotitis, whether the presenting symptoms are of the "inflammatory obstructive" or "tumor-simulating" type.

Similarities between the microscopic pathology in the salivary gland in Mikulicz's or Sjögren's syndromes and that in the thyroid in Hashimoto's disease have been alluded to by some authors (30, 32); indeed, the concurrence of Hashimoto's thyroiditis and Sjögren's disease has been noted (36). An immunological similarity may also exist, since in Hashimoto's disease serum antibodies against thyroid tissue have been reported (37), and Jones (38) has found lacrimal and salivary gland precipitating antibodies in patients with Sjögren's syndrome.

The cases we report, together with those described by others and referred

to in the introduction, demonstrate that enlargement of salivary glands may be an accompaniment of systemic disease other than sarcoid and neoplasms. They lend support to the validity of conducting a careful search for other manifestations of disease in patients who present themselves with persistent salivary gland swellings not due to neoplasm.

SUMMARY

1. Ten subjects are described in whom enlargement of salivary glands, usually bilateral, was accompanied by signs and symptoms of a systemic disease.

2. Frequently present were manifestations of probable disease of the lacrimal glands.

3. Abnormalities, aside from those in the salivary and lacrimal glands, varied and included lymphadenopathy, splenomegaly, purpura, hemolytic anemia, hyperglobulinemia, arthralgia, rheumatoid arthritis, and probable lupus erythematosus and scleroderma.

4. Eight salivary glands were biopsied; pathologic findings included lymphoid infiltration and reduction in acini.

ADDENDUM

The reader's attention is invited to the report by Heaton (39) presenting his evidence for common etiological relationships in Mikulicz's disease, Sjogren's syndrome, lupus erythematosus, Felty's syndrome, and Hashimoto's thyroiditis.

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THE CYTOARCHITECTURE OF THE POSTCENTRAL GYRUS OF THE MONKEY MACACA MULATTA[1]

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INTRODUCTION

A knowledge of the functional significance of morphological differences is essential for an understanding of the physiology of any part of the nervous system. This is particularly true of the cortex of the cerebral hemispheres. That detailed differences in the cortical cytoarchitecture exist is well known, and these differences have formed one of the bases for its subdivision into numerous areas. Although the meaning of such differences is still obscure, it has been possible to make certain correlations with afferent connections of the cortex. Thus for at least some cortical regions it has been established that areal boundaries defined on the basis of architectonic characteristics coincide with the limits of the projection areas of specific thalamic nuclei, determined by the method of retrograde degeneration (13, 14, 15). Furthermore, the boundaries of the primary sensory areas for tactile and auditory sensibility determined in another way—by the use of the evoked potential method—correspond with reasonable accuracy to those established by the architectonic method (1, 15, 20). While many important problems remain, particularly with regard to regions of transition between cortical areas that are clearly different from one another, the general agreement achieved by the use of these three methods for defining these two specific receiving areas lends considerable authority to the definitions which are already established, and which are in common usage.

Cortical structure is, however, not uniform within either of these sensory regions, and architectonic subdivisions of them have been described. The question therefore arises concerning the functional importance of such anatomical differences within a single sensory area as a whole. Rose and Woolsey (12, 15) have previously concerned themselves with this problem. In their combined anatomical and physiological investigations of the auditory region of the cat they established four subdivisions based upon morphological differences: a central region, auditory I, surrounded by a belt divisible into three areas, the suprasylvian fringe sector, the posterior ectosylvian area, and the auditory II. Woolsey and Walzl (19), however, indicated that while the total

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cortical area responsive to electrical stimulation of cochlear nerve fibers in deeply anesthetized cats corresponded well with the *whole* auditory area defined morphologically, only two subdivisions could be distinguished on functional grounds, auditory areas I and II. The possible functional implications and the problems raised by these findings were discussed by Rose and Woolsey.

Evidence that the three subdivisions of the somatic sensory cortex of the macaque monkey, described by Brodmann (4) are likely to have important functional differences derives from the experimental anatomical study of Le Gros Clark and Powell (7). They found that each of the three subdivisions of the monkey's postcentral gyrus (areas 3, 1 and 2 of Brodmann) possesses differential afferent connections from the posterior ventral nucleus of the thalamus.

It is to this particular problem of the functional meaning of the structural differences within a primary sensory receiving area of the cortex that we have addressed ourselves. We have combined the methods of cytoarchitectural study and of single unit analysis in an attempt to determine whether the architectural differences within the somatic sensory cortex of the macaque monkey can be correlated with differences in the functional organizations of the three areas of which it is composed.

In the present paper the cytoarchitecture of the medial (or upper) two-thirds of the postcentral gyrus is described. This description is given not only to serve as the anatomical basis for the physiological findings presented in a second paper but also because of discrepancies in previous descriptions of this region. Brodmann (4), the Vogts (16), Peele (10) and Krieg (5) all recognized three subdivisions, areas 3, 1 and 2 respectively from before backwards, but Bonin in *Cebus* (2) and Bonin and Bailey in *Macaca* (3) could not distinguish a separate area 2; and in a study of *Ateles* and *Macaca* Lashley and Clark (6) were unable to distinguish any subdivision at all within the postcentral region.

MATERIAL

Blocks of the pre- and postcentral gyri of 19 hemispheres of immature macaque monkeys were used in the present study. The hemispheres were selected from the 27 brains investigated in the physiological experiments: apart from slight glial reaction in some brains the exposure of the cortex during the experiment did not appreciably affect the histological appearance, as reference to the illustrations will demonstrate. The brains were fixed in 10 per cent formalin, embedded in paraffin wax and sectioned at 20μ. The blocks were cut either in the saggital plane or in a plane at right angles to the greater extent of the central sulcus. For some brains the plane of section was determined, at the end of the experiment, by inserting guide wires for sectioning in the exact plane and angle of entry of the penetrating microelectrodes. Every effort was exerted to make this angle normal to the surface of the cortex. One additional block

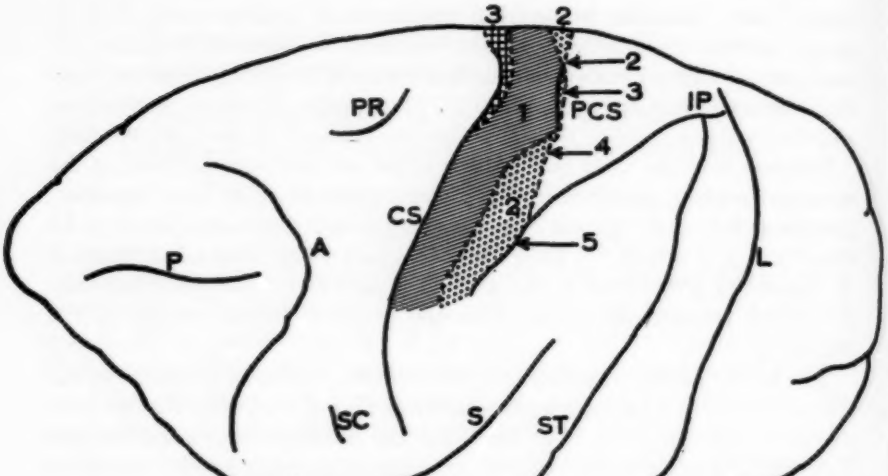

FIG. 1. Diagram of a reconstruction of the extent of the three areas of the somatic sensory cortex of Monkey PPC 7. Outline of hemisphere and sulci traced from a photograph of the brain. Numbers with arrows to the left indicate positions and planes of section of Figures 2–5, and of the photomicrographs of Figures 6–11. Numbers without arrows indicate the cytoarchitectonic areas. Abbreviations as follows: A—Arcuate Sulcus; CS—Central Sulcus; IP—Intraparietal Sulcus; L—Lunate Sulcus; P—Principal Sulcus; PCS—Postcentral Sulcus; PR—Superior Precentral Sulcus; S—Sylvian Fissure; SC—Anterior Subcentral Sulcus; ST—Superior Temporal Sulcus.

from an animal not used in the experiments was cut horizontally. All the sections were mounted and stained with thionin. The preparation of serial sections was required primarily for finding the electrode tracks, but they proved to be useful in the architectonic study as well; thus comparison of adjoining sections made it possible to define clearly what were only minor and inconstant changes within a field, and also to gain an impression of their frequency. It should be emphasized that our study of the postcentral cortex is limited strictly to that portion lying between the medial margin of the hemisphere and the lateral end of the intraparietal sulcus (Fig. 1).

RESULTS

Cytoarchitecture of the postcentral gyrus

Rose (12) has recently suggested a new concept of an architectonic field, and because this has proved of great value in our study of the postcentral

gyrus the relevant points will be mentioned; for a fuller discussion the original papers should be consulted (12, 14). In the early work on cytoarchitecture it was generally held that a given field was uniform in structure, and minor variations within such a field were disregarded. Later research, however, indicated that the minor variants could not be ignored, and they were indeed utilized by some authorities to subdivide even further the main cortical areas. From studies on the correlation of cortical architectonic structure with the area of projection of specific thalamic afferent fibers, Rose concluded that such variants should be grouped together, a natural series constituting a field. With this concept a cortical field is not generally morphologically uniform, but displays constant changes in structural detail; changes which vary systematically were designated gradients of a field. The important conclusion is that two fields which have definite overall differences may yet have no sharp border between them.

In the cortex of the postcentral region there is a definite antero-posterior gradient of morphological change, but as the gradient is not uniformly gradual three fields may be recognized (Fig. 1). The anterior field, area 3, is clearly distinguishable; posterior to it is a broad area of gradual change within which two subdivisions, areas 1 and 2, having significant overall differences may be differentiated. The most important single feature which distinguished areas 1 and 2 from each other and from areas 5 and 7 is the variation in the number and size of the large pyramidal cells in layers III and V. Since these cells are, in general, slightly larger and more conspicuous in the medial third or so of the gyrus, i. e., medial to and including the postcentral sulcus, the differentiation of these two areas is clearer in this part of the gyrus (Fig. 10).

The extent and limits of the three areas have been found to vary slightly in different brains, probably due partly to slight differences in the planes of section and possibly in part to actual individual differences.

The topography and structure of area 3 (figs. 1–8)

In most brains this area is exposed on the surface of the brain only in the medial part of the postcentral gyrus where the central sulcus is either quite shallow or completely absent. Over the rest of the gyrus it forms the posterior wall of the sulcus. The junction with the cortex of area 4 anteriorly is not sharp, but is marked by a short area of transition labelled 3a by the Vogts (16) and PA by Bonin and Bailey (3). For the most part this transitional area is situated at, and slightly behind, the bottom of the sulcus; laterally, where the bottom of the sulcus is often broad and triangular on section, area 3a forms most of the floor. At the medial end of the sulcus both areas 3 and 3a come to occupy, in addition, the deepest third or so of its anterior wall.

The cortex of area 3 is the most characteristic and clearly differentiated area in the postcentral gyrus. The thinness of the cortex is in marked contrast to

the thickness of that in area 4 on the anterior wall of the sulcus. In area 3 layers II, III, and IV are not clearly demarcated from each other but blend to form one broad lamina. The degree of fusion of the layers varies in different parts of the field, being most marked in the middle third of its antero-posterior extent and becoming progressively less towards the boundaries with areas 3a and 1, respectively. All three layers are densely packed with small cells, but the absence of clear lamination is largely due to the structure of layer III. Here, in addition to the small pyramidal cells, there are many granules and small polymorphic cells. Over most of its extent the layer is uniformly dense and no division into sublayers is present. Towards the anterior and posterior boundaries of the field gradual changes occur: the granule cells become progressively fewer and the superficial part of the layer less dense. No gradient of cell size between the superficial and deep margins of the layer is evident, but at the junction with layer IV there is a thin, irregular layer of medium sized pyramidal cells. These cells do not stain deeply, are plump rather than sharply triangular, and are not at all conspicuous. Clusters of larger, deeply staining pyramidal cells in the deeper part of the layer are seen in occasional sections. Layer II is densely packed with small and medium sized granules, and it tends to become slightly thicker in the middle third of the area. In layer IV the small granules predominate, but medium sized polymorphic cells and an occasional large pyramid are seen. The sparsely populated layer V stands out as a relatively clear undivided strip compared with layers II, III, and IV. The majority of the cells are small pyramids, but in the superficial part, underlying layer IV, are some medium sized pyramids. These cells are of approximately the same size as those found in the deepest part of layer III, but in contrast to the latter they are deeply staining and distinctly triangular in shape. Large pyramidal cells are rarely seen. Layer VI is not divisible into two layers and the boundary with the white matter is sharp: it is quite richly filled with small and medium sized polymorphic and triangular cells. In general a columnar arrangement of the cells is not well marked in area 3. There is, however, definite variation between different parts of the gyrus and between different brains, and not infrequently distinct columns are present in the anterior third of the area.

In the transition zone, between areas 3 and 4, area 3a, there is progressive thinning of layer IV; in addition, the gradual reduction in the cell density and in the number of granule cells in layer III, which began in the lower third of area 3, is accentuated.

The structure of area 1 (figs. 1–8)

More than one difference distinguishes this from area 3. Some of the changes begin very gradually in the posterior third of area 3, and, after an abrupt increase close to the posterior bank of the central sulcus, they continue gradu-

Figure 2

Figure 3

Figure 4

Figure 5

FIGS. 2–5. Drawings of saggital sections of the postcentral gyrus made from tracings of photomicrographs taken at positions indicated in Figure 1. Numerals indicate cytoarchitectonic areas. Dotted lines indicate boundaries of areas.

ally through the antero-posterior extent of the gyrus. The anterior limit of the area is thus clearly marked by the sudden changes close to the central sulcus, but the transition to area 2 posteriorly is gradual. At the level of the postcentral sulcus this transition from area 1 to area 2 usually occurs around the bottom of the sulcus, but it may be found more anteriorly on the anterior wall of the sulcus. Medial to the postcentral sulcus, area 1 forms a strip on the lateral surface of the gyrus immediately behind the posterior bank of the central sulcus. More laterally this area gradually increases in width until it covers the posterior bank of the central sulcus and rather more than the anterior half of the lateral surface of the gyrus.

The most marked differences between area 3 and area 1 are found in layer III. The constituent cells of this layer all become pyramidal in shape and show a size gradient between the superficial and deep margins. The majority of the cells are small or medium sized pyramids, but in the deeper third of the layer large, deeply staining pyramids appear. In the anterior part of the area these larger cells tend to be arranged in clusters, but posteriorly they increase in number to form a narrow sublayer 2–3 cells thick, III C. The reduction in cell density of this layer which began in area 3 is accentuated, particularly in the superficial half. With the convergence of these changes in layer III the differentiation of layers II, III, and IV becomes clearer. Layer II is less densely packed with small granule cells than in area 3, and the presence of a few small pyramids is another distinguishing feature. Similarly, layer IV is less dense here than in area 3, but because of the large pyramids immediately superficial to it and the lighter layer V it stands out more sharply. Most of the polymorphic cells have been lost, only an occasional one remaining amongst the small granule cells making up the layer. As has been mentioned, layer V becomes lighter, having a low density of small and medium sized pyramids. In the superficial half of the layer, however, there are more large pyramids of about the same size, but rounder in shape than those in IIIc. These large pyramids increase in number in the posterior parts of the area but are clearly less numerous than those in IIIc. Coincidently with the changes in the other layers close to the posterior bank of the central sulcus, layer VI thickens and divides into two layers. The cells are small and medium throughout the entire thickness of the layer, but the cell density is considerably less in the deeper sublayer. In addition, the cells stain less deeply there, and consequently the separation of layer VI from the white matter becomes less sharp. A constant and striking feature of the structure of area I is the *pronounced columnar arrangement of the cells*, affecting all six layers.

Structure of area 2 (figs. 1–6; 8–11)

Neither the anterior nor the posterior border of this area is sharply defined. Medial to the postcentral sulcus, and approximately in line with it, area 2

covers a small triangular area of the lateral surface of the gyrus. At the level of the postcentral sulcus, where the cortex of area 2 is largely buried, it usually forms the posterior wall and bank and frequently a varying amount of the anterior wall of the sulcus. Lateral to the postcentral sulcus area 2 is initially found only on the exposed surface of the hemisphere, but eventually it forms, in addition, the anterior bank and wall of the intraparietal sulcus. Immediately posterior to area 2 in the greater part of its extent is the cortex of area 5, but laterally, where area 2 occupies the anterior wall of the intraparietal sulcus, area 7 is directly behind it.

An increase in the number of large pyramidal cells in the deeper part of layer III and in layer V marks the transition between areas 1 and 2. In layer III the large pyramidal cells come to form quite a compact layer three to five cells thick. These cells are no larger than in area 1, but they tend to be plumper and less triangular in shape. The superficial half of layer III becomes slightly less dense, and consequently both the subdivisions of this layer and the demarcation of layers II and III become sharper. The large deeply staining pyramids in the superficial part of layer V show an increase in size as well as in number; the changes in this layer, however, are less marked in the lateral part of the gyrus. In the posterior part of the area an occasional large pyramidal cell, of the same order of size as the giant pyramidal cells of the motor cortex, is seen. Layers II and IV show little appreciable change, but they are both more clearly demarcated from the adjacent pyramidal cell layers. The transition from area 1 to area 2 is indicated also by an increase in the thickness of the cortex, and while all layers share in this increase it is relatively greater in layer III. The thickness of that part of the cortex of area 2 occupying the anterior wall of the intraparietal sulcus, however, is considerably reduced.

The transition to either area 5 or area 7 is also gradual. The changes in the third layer are the most striking, where there is a further reduction in the density of the small pyramids in its superficial part, and of the large pyramids in the deeper part. Similarly, the large pyramids in layer V progressively decrease in number, but in this layer in area 5 some giant Betz-like cells are seen. Layers II and IV are sharply demarcated, and the increase in clarity of lamination which commenced at the posterior bank of the central sulcus attains its maximum here. A further distinguishing feature is the pronounced columnar arrangement of the cells, particularly in area 7.

DISCUSSION

In the somatic sensory cortex of the macaque monkey three areas may be recognized, a well defined sector of koniocortex, area 3, and posterior to it areas 1 and 2 together forming a broad area within which the morphological characteristics change gradually to the homotypical cortex of the parietal lobules. It will have been noted that we have described some of the architectonic

characteristics as varying systematically in an antero-posterior direction—not only within a field but throughout the extent of the postcentral gyrus—and that the differentiation of the gyrus into three areas is based upon such gradients of change. In general it may be stated that, if the cortex is traced in an anterior direction from any point within area 2 or area 1 the cytoarchitectonic structure will become progressively more granular, layers II, III, and IV will become blurred, and there will be a diminution in the number of large pyramidal cells in layers III and V. When the cortex is traced in a posterior direction the converse changes are seen. If one assumes—and there is now good evidence for doing so—that architectonic characteristics have functional significance, then one would expect a similar gradation in fuctional properties within the postcentral gyrus. In the paper which follows experimental evidence will be presented which confirms this prediction.

The most anterior field, area 3, does not show the pronounced development of the granular layers found in the visual cortex, but it does have all the features of koniocortex as broadly defined by Rose (12) in his study of the auditory cortex of the cat. In both the auditory and the somatic sensory primary receiving areas layers II, III and IV are not clearly demarcated from each other and have a high cell density. In all layers little variation in cell size is found, and most of the cells are small. The third layer in area 3 may be considered to be particularly characteristic in having a large number of granule cells in addition to the pyramidal cells customarily found in this layer.

The reason for the basically similar structure of the primary sensory areas is not known. Rose (12) has suggested that the characteristic high cell density may be correlated with the very dense thalamic projections to these areas. Relevant to this is the evidence from experimental anatomical studies that area 3 receives a higher density of thalamic afferents than do areas 1 and 2 (7, 11, 18). In the second paper physiological observations in accord with these anatomical findings will be described.

Beginning in the posterior part of area 3 and continuing throughout areas 1 and 2 the cell density in layers III and V progressively diminishes, and all the cells become pyramidal; as a result of these changes the laminar pattern of the cortex becomes more distinct. The border between areas 1 and 2 may not be sharp, but when the structural arrangement of each area is considered as a whole the differences are impressive. The most striking of these differences is the increase in number of the large pyramidal cells in layer III of area 2. These changes cannot solely be due to the fact that area 2 is to a large extent buried in the walls of sulci, as Bonin and Bailey (3) have suggested, for they are equally well marked where areas 1 and 2 lie on the exposed surface of the gyrus. Further, at the level of the postcentral sulcus, where the junction of these two areas is commonly found at the bottom of the sulcus, the structure of the buried cortex of the anterior and posterior walls is strikingly different.

It is extremely unlikely that such differences are the result of cortical folding alone; it is well known, on the other hand, that sulcal formation frequently occurs at the junction of different architectonic areas. The fact that the bottom of the sulcus is not constantly the site of the boundary between areas 1 and 2 is not surprising. Such variations occur elsewhere also. For example, the central sulcus is shallow at its medial end, and there the koniocortex of area 3 extends onto its anterior wall.

In any discussion of the descriptions of the postcentral region by different authors it should be borne in mind that the microscopic appearance of the cortex is appreciably altered by differences in technical preparation, and that even slight differences in the planes of section may considerably affect the ease with which boundaries of areas are defined.

Our map of the somatic sensory cortex and the criteria used for distinguishing the three areas agree closely with the original description of Brodmann (4) with that of the Vogts (16), and with that of Krieg (5). Brodmann, however, described area 2 as existing only within the walls of the postcentral and intraparietal sulci, whereas we have found it also on the exposed surface of the gyrus above and immediately below the postcentral sulcus. Our description of the postcentral gyrus of the macaque monkey is very similar to that of the same region of the human brain by M. Vogt (17). Peele (10) also identified three areas in the postcentral gyrus of the macaque monkey and described this area as extending more anteriorly than we do.

Le Gros Clark and Powell (7) in their experimental investigation of the postcentral gyrus did not make a detailed study of the architectonic structure of the cortex, but they found that the three areas described by Brodmann have differential connections from the posterior ventral nucleus of the thalamus. These findings are in agreement with other experimental work which has demonstrated that changes in cytoarchitecture can be correlated with differences in thalamic projection, and that the boundaries of a cortical area determined on the basis of morphological detail are coextensive with the areas of projection of specific thalamic nuclei, (13, 14, 15). In a subsequent paper evidence from electrophysiological experiments is presented by us which indicates that the architectonic subdivisions of the postcentral gyrus also have a functional significance.

Bonin (2) in *Cebus* and Bonin and Bailey (3) in *Macaca* failed to distinguish a separate area 2. These authors describe only two areas in the somatic sensory cortex (PB and PC), but they have subdivided off area PEm from the anterior parietal cortex. They state in the text that the extent of this area PEm agrees closely with Brodmann's area 5, but from their maps it is clear that PEm more closely coincides with Brodmann's area 2. PEm is said to be distinguished by the giant pyramidal cells in layer V, but their photograph showing these cells is almost certainly taken from area PE (compare their frontispiece map with

figure 4). It is of interest that the photograph of PC (which from a comparison of the same two figures can be seen to extend into area PEm) shows, in our opinion, the characteristics of areas 1 and 2; thus the left (anterior) half of their photograph has the characteristics of area 1, and the right (posterior) half shows the features of area 2, particularly because of the larger pyramidal cells in layers III and V. It appears, therefore, that the discrepancy between these two descriptions is more apparent than real, and that Bonin and Bailey have stressed unduly the minor discrepancies.

It is difficult to understand the failure of Lashley and Clark (6) to distinguish any subdivision within the postcentral gyrus, because the koniocortex of area 3, at least, is quite distinct. Although they describe the cortex of the postcentral gyrus as "remarkably uniform" (p261) they later state (p293) that "The caudal wall and lip of the central sulcus are generally distinguishable from the convexity of the postcentral gyrus". These authors also failed to distinguish any subdivisions within the cingulate cortex of *Ateles* and *Macaca*. In the limbic cortex of the rabbit and cat, however, Rose and Woolsey (14) have shown that three areas can be defined, not only on their morphological characteristics, but also on the basis of their different thalamic connections. Rose and Woolsey consider that Lashley and Clark "have exaggerated some justifiable objections, and they have unfortunately included the limbic cortex in their all too sweeping generalizations". These comments apply with equal force to Lashley and Clark's interpretation of the somatic sensory cortex.

A striking and constant feature of the cortex of areas 1, 5, and 7 is the pronounced columnar arrangement of the cells. This arrangement is less marked in area 2, and in area 3 is inconstant, varying not only between different brains, but even between different parts of the same hemisphere. The different positions of the cortical areas showing pronounced columnar arrangement of the cells indicates that this morphological feature is not necessarily related to cortical folding (cf. 6). Thus it is found in area 1 on the posterior bank of the central sulcus, in area 5 on the lateral surface of the gyrus and in area 7 on the posterior wall of the intraparietal sulcus. This suggests that the degree to which the cells are arranged in columns in any histological section of the neocortex may depend, to a large extent, upon the precise plane of section. However, a detailed study of extensive material would be necessary before one could reach a conclusion on this point. The significance of this morphological arrangement lies in the recent findings of Mountcastle (9) and confirmed in the present series of experiments, which indicate that such a vertically linked group of cells is likely to serve as an elementary unit of cortical function.[3] It may be noted that the cells of another primary sensory area, the auditory cortex, are arranged in well-defined columns.

[3] Lorente de No (8) concluded from his studies of cortical sections stained by the Golgi technique that structurally such a functional unit consists of a specific thalamo-cortical fiber

SUMMARY

The cytoarchitecture of the medial two-thirds of the postcentral gyrus of the macaque monkey is described. Three fields are recognized. The anterior field area 3 is composed of koniocortex, and areas 1 and 2 are formed of cortex showing graded changes to the typical parietal cortex of areas 5 and 7. Area 2 is characterized by the increase in number of large pyramidal cells in layers III and V. This analysis has been discussed in relation both to the previous descriptions and to the experimental results on the thalamic projection to this region.

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and the cylindrical group of cortical cells which surround the termination of the fiber; some of the cells are in direct synaptic relation with the fibers, but others are not. We can not define in any quantitative manner the size of the functional unit, except to say that it undoubtedly consists of a group of fibers rather than a single fiber.

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PLATE I, FIG. 6. All photomicrographs of this and the following plates are of sections of the left hemisphere of monkey PPC 7; sections cut at 20 μ after embedding in parrafin, and stained with thionin. Arrows indicate boundaries of areas, and the cortical layers are denoted by Roman numerals. Details of structure are described and discussed in the text.

Postcentral gyrus at level of Figure 3, × 20.

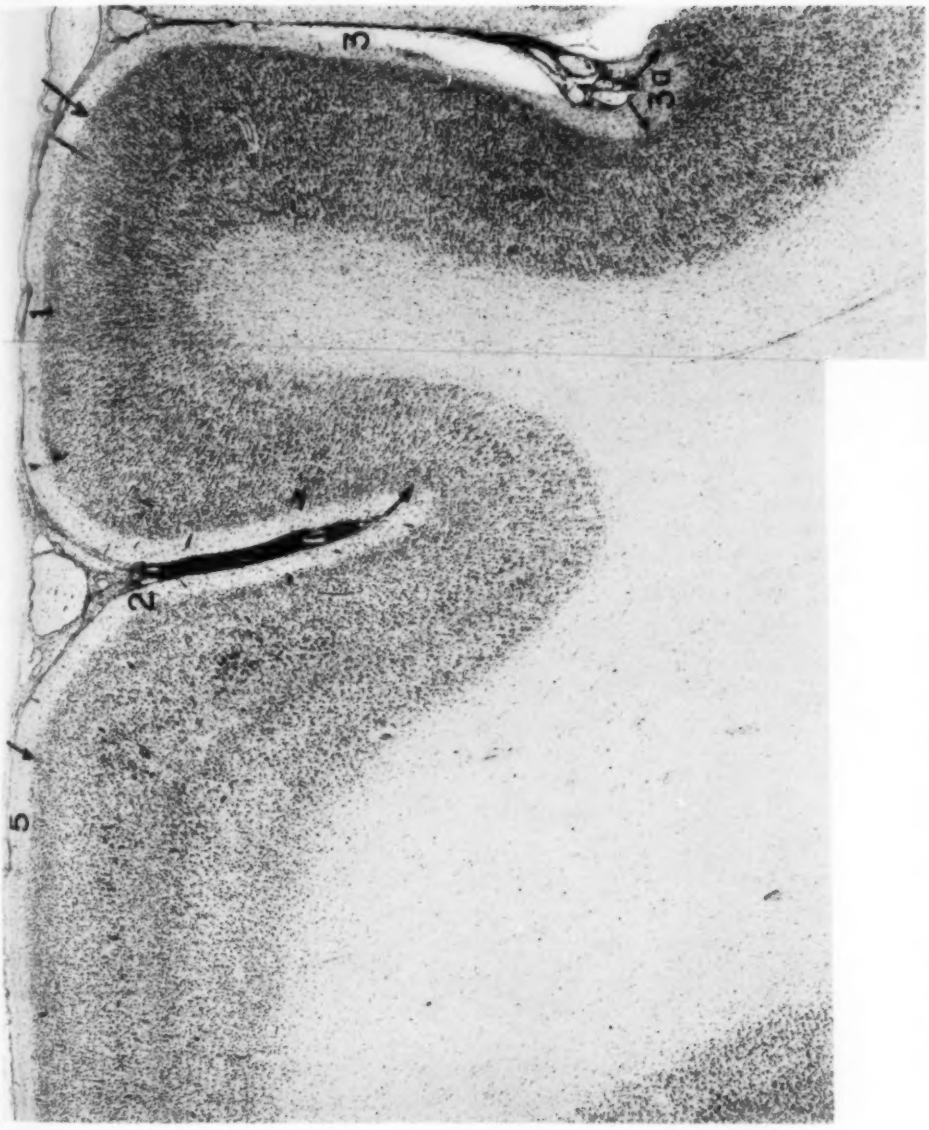

3
3a
1
2
5

PLATE II, FIG. 7. Posterior wall and bank of central sulcus at level of Figure 5 to show change in structure between areas 3 and 1. Note blurring of layers II, III and IV and general smallness of cells in area 3, and in area 1 the marked columnar arrangement of the cells, clearer demarcation of layers II, III and IV, and larger pyramidal cells in layers III and V. × 37.

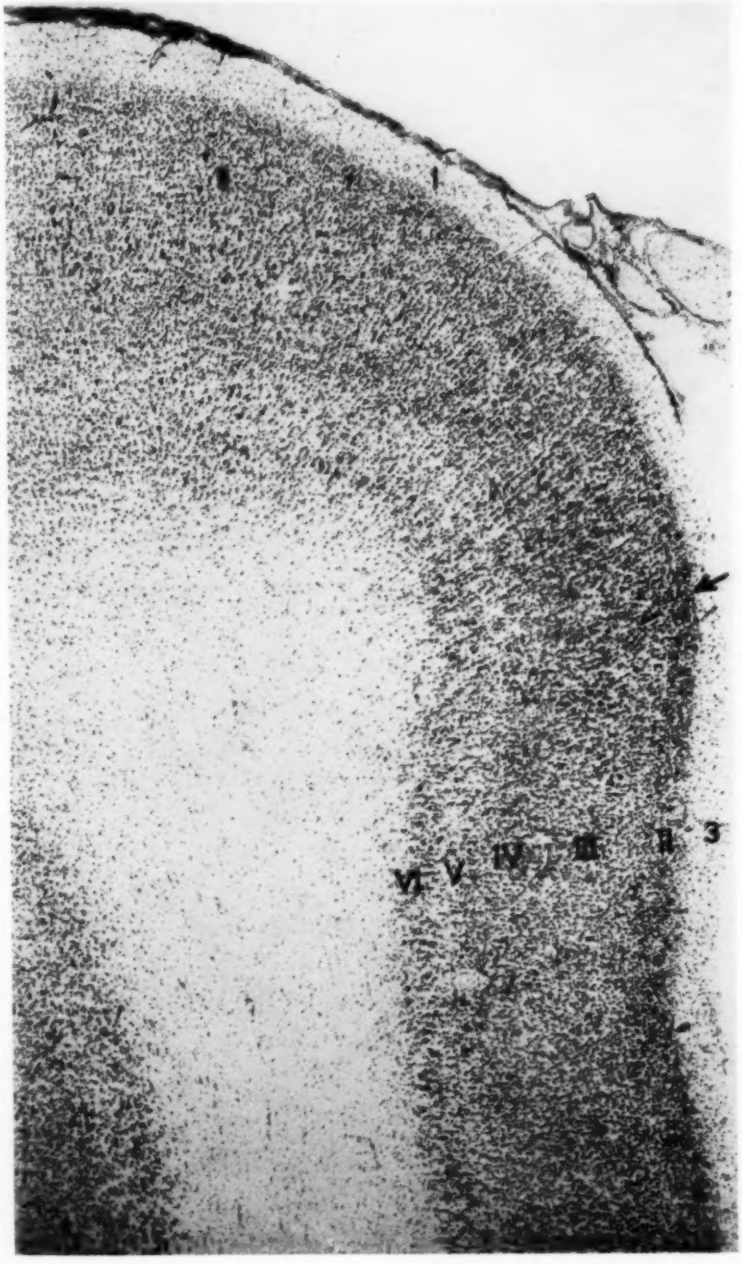

VI
V
IV
III
II
3

PLATE III, FIG. 8. Posterior bank of central sulcus and adjoining lateral surface of postcentral gyrus at level of Figure 4. The characteristic features of each area can be seen, and also the gradual increase in size of the pyramidal cells in areas 1 and 2. *x* marks identical position on section shown in this and the photomicrograph of Figure 9. × 37.

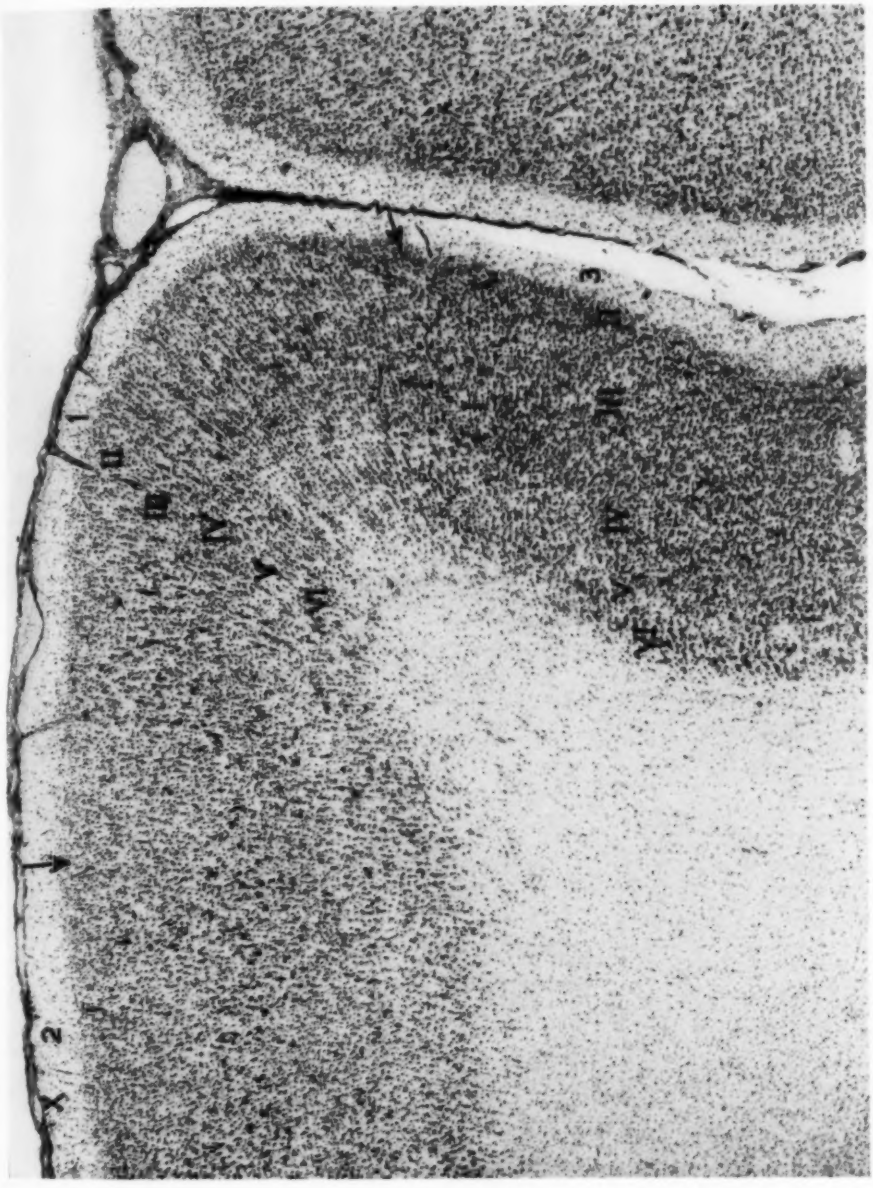
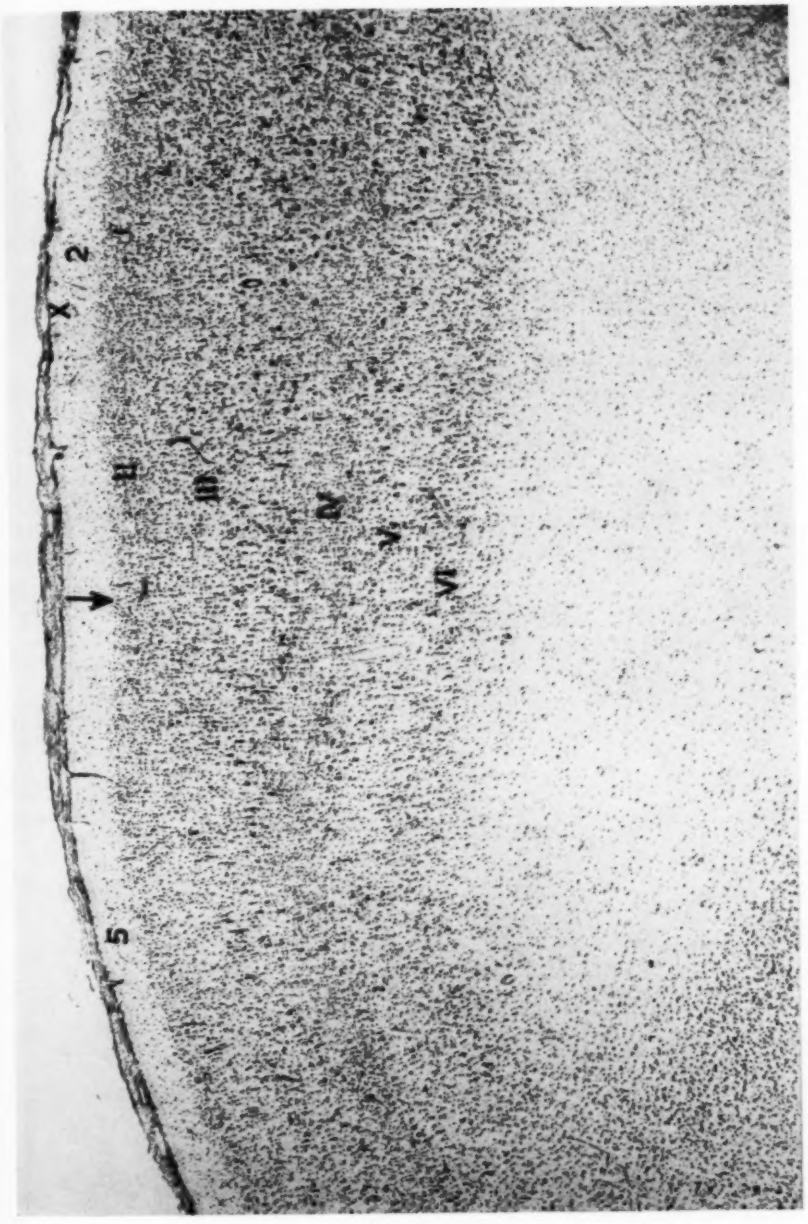

I
II
III
IV
V
VI
2

PLATE IV, FIG. 9. Continuation posteriorly of the section shown in Figure 8. The photomicrograph shows the transition from area 2 to area 5; × 37.


2
I
II
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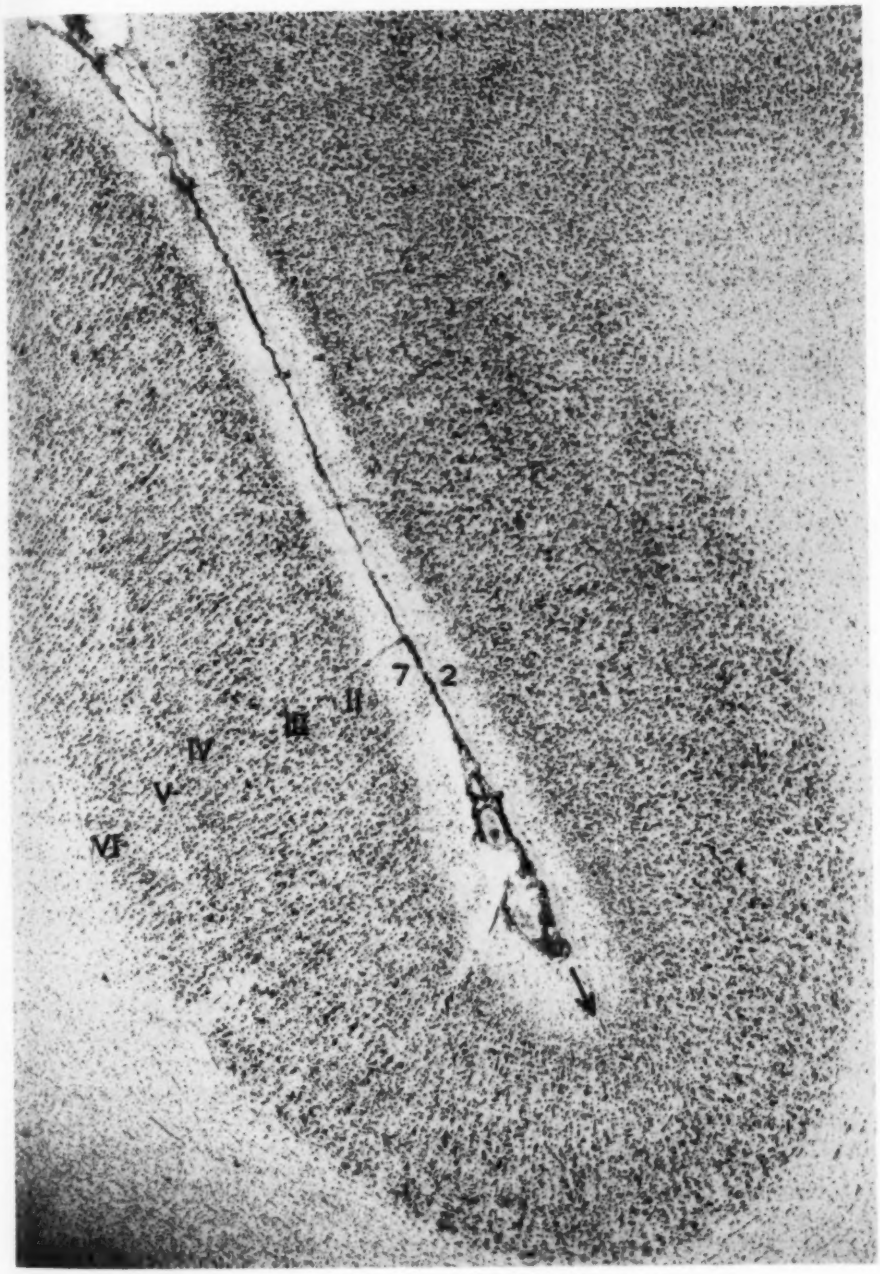
VI

PLATE V, FIG. 10. Postcentral sulcus at the level of Figure 3. Note the large pyramidal cells in layers III and V of area 2, and the occasional Betz-like pyramidal cell in layer V of the posterior part of area 2 and in area 5; × 35.

PLATE VI, FIG. 11. Intraparietal sulcus at level of Figure 5. Note the distinct lamination and columnar arrangement of the cells in area 7; × 37.


7
2
II
III
IV
V
VI

SOME ASPECTS OF THE FUNCTIONAL ORGANIZATION OF THE CORTEX OF THE POSTCENTRAL GYRUS OF THE MONKEY: A CORRELATION OF FINDINGS OBTAINED IN A SINGLE UNIT ANALYSIS WITH CYTOARCHITECTURE[1]

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INTRODUCTION

In this paper we wish to describe in part the results of a study of the postcentral gyrus of the macaque monkey, using the method of single unit analysis, and to indicate in what way and to what an extent correlations can be made between the detailed morphology of this cortical region and the functional properties of its constituent neurons. This investigation is an extension to the primate cortex of an earlier and more limited study of the first somatic cortex of the cat, the homologue in carnivores of the primate postcentral gyrus, which was carried out without a detailed histological study of the experimental brains (9, 10). The results obtained in the present study, presented in Part I, do in fact show a correlation between the cytoarchitectural gradient across the postcentral gyrus, described in the preceding paper (15), and the gradient of functional properties of the cortical cells.

From the earlier study in the cat alluded to above several lines of evidence were adduced which support an hypothesis of the functional organization of the somatic sensory cortex. That is, that the neurons lying in narrow vertical columns extending from layer II through layer VI make up an elementary unit for function. This hypothesis was based upon the facts that neurons of such a vertical column are activated by the same single submodality of somatic sensibility, from almost identical peripheral receptive fields, at latencies not significantly different for the cells of different layers. The results of the present series of experiments fully support this hypothesis for areas 1 and 2 of the postcentral gyrus of the monkey. Technical difficulties have prevented the collection of wholly convincing data for area 3, but all the data which have been obtained indicate that it holds for that region also. These results are presented in Part II of this paper.

In the monkey we have observed large numbers of cortical neurons which are selectively sensitive to the steady position or angular displacement of the

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joints of the limbs. These data are described in the following paper (11) and a synthesis is presented of the neural mechanisms which appear to be the functional substratum of what is commonly called the sense of position and movement.

In the last paper of this group (12) the results of our studies of those cortical neurons selectively sensitive to cutaneous stimuli are brought together. These lead, in a preliminary synthesis, to a description of the central neural events set in motion by peripheral stimuli which can properly be called tactile in nature. Particular emphasis is placed upon the role of afferent inhibition in sensory tactile discriminations.

METHODS

Experiments were made upon 27 immature macaque monkeys, whose body weights ranged from 4.0 to 7.0 kg. The experiments were numbered consecutively, as were the microelectrode penetrations made in each experiment. The neurons observed in each experiment were lettered consecutively, and those whose discharges were photographed were given numerals as well. Thus, in the text which follows, such terms as M7-P3 and 9-22 would refer respectively to the third penetration of the seventh experiment, and to the twenty-second neuron observed and studied photographically in the ninth experiment; 11-aa would refer to the 27th neuron observed in the eleventh experiment, and indicate that no photographic records of its discharges were made.

Initially anesthesia was induced by the intravenous injection of 20–25 mg./kg. of sodium thiopental. As the resulting anesthetic state lightened (in 1–2 hours) a regime of intermittent intravenous injections was begun, consisting of 10–15 mg. of sodium thiopental alternating, usually at half-hourly intervals, with small quantities of a neuromuscular blocking agent, commonly 1 mg. of d-tubocurarine chloride, but in later experiments 10 mg. of gallamine triethiodide (Flaxedil®, American Cyanamid). Respiration was maintained by a pump when necessary. The intermittent recovery from complete neuromuscular block which occurs with this regime allows one to assay the anesthetic state at regular intervals, and to adjust the subsequent quantities of the barbiturate injected. The animals were maintained in the unconscious, lightly barbitalized state at all time; spontaneous and reflexly evoked movements were prevented for a large part of the time by the blocking agent. The very active state of the cerebral cortex under these conditions and the ease with which its neurons can be brought to action by sensory stimuli have been described in an earlier paper (9) The deep body temperatures of the animals were maintained in a narrow range around 38 degrees C. Small quantities of 0.9 per cent NaCl solution were given with each injection of a drug, and these appeared sufficient to maintain fluid balance, for urination occurred spontaneously every 3 to 4 hours. In this way the animals remained in good condition, and the experiments were continued for from 24 to 36 hours.

Method of Recording. The microelectrodes were similar to those described earlier by Dowben and Rose (5), and used in several previous investigations (9, 10, 17). The electrode is a glass pipette drawn with a very gradual taper to a tip of 2 to 4 μ diameter. The electrical properties of somewhat similar electrodes have been described by Svaetichin (21). The electrodes were passed through small openings in the pia-arachnoid, made by microdissection, and into the cortical tissue by means of a Davies microdrive operating through a closed cortical chamber (4). The details of the method, and the stability of single unit recording in

the cortex achieved by thus re-establishing normal hydraulic conditions within the cranium have been described earlier (10).

Amplification and Display of Responses. The potential differences taking place between the microelectrode tip and the headholder were led to a cathode follower circuit driving a Tektronix model 122 differential, capacity-coupled, low level pre-amplifier. The amplifier output (or of two driven in parallel) was displayed by a two beam cathode ray oscilloscope, and photographic records were taken with a Grass Kymograph camera. The overall frequency response of the system tested with a typical microelectrode in the brain tissue, shows 3 db drop points at 14,000 cycles per second and at 10 cycles per second for the high and low ends, respectively. Variable high and low band pass filters were available. In general records were made with the low frequency response limited to about 200 cycles per second (short time constant recording—STC). Occasionally the two paralleled pre-amplifiers were operated together, one at LTC and the second at STC, and their outputs viewed simultaneously. The recording parameters for the records shown in the illustrations of this and the following papers are indicated in the legends. The oscilloscope used provides a wide range of display patterns, sweep expander properties, etc.

Methods of Stimulation. Peripheral receptive fields of neurons activated by stimulation of the skin were mapped by using light mechanical stimulation with a small rod, or a small camel's hair brush. In this way neurons driven by hair movement can be distinguished from those responsive to pressure upon the skin. One can also determine with some certainty the rate of adaptation of the response of a cortical neuron to steadily applied cutaneous pressure. For more accurate timing of events electrical stimuli were delivered through a pair of sharp needles, mounted about 1 mm. apart and insulated to their tips, which were thrust into the skin. The stimulating and timing circuits provided a wide range of pulse and train sequences and of stimulus intensities and durations. It seems reasonable to us to assume that this form of stimulation excites the terminal branches of cutaneous afferent fibers, rather than the nerve endings themselves. With the very short stimulating pulses used, usually 0.1 msec., it seems likely that changes in stimulus strength produce changes in cortical nerve cell discharge by increase or decrease in the number of active fibers, and not by evoking more than one impulse per stimulus per fiber. In spite of its obviously abnormal temporal and spatial pattern of input, this form of stimulation, in our experience, evokes patterns of discharge in cortical neurons which resemble rather closely those evoked by brief mechanical stimuli delivered to the skin.

Histological Studies. The majority of the electrode tracks were extremely difficult to find, and meticulous attention to detail at each of the stages to be described was essential. During the experiment a photograph of the exposed area of the cortex was taken through the dissecting microscope. This was developed and a print made (at about 10× magnification) before the first electrode penetration was made. The precise position of the insertion of each of the electrodes in relation to adjacent arteries and veins was marked on the photograph when the opening in the pia-arachnoid was made. After fixation, the dorsolateral surface of the hemisphere was photographed.

The blocks of the postcentral gyrus of the brains used in the first 10 experiments were cut in the sagittal plane. As the electrode penetrations were at a slight angle to the sagittal plane, however, the tracks made in these experiments were cut tangentially and only small segments of the tracks appeared on successive sections. It was difficult to distinguish the appearance of these tangentially cut tracks from small blood vessels or minute foci of gliosis. An electrode track, however, could be traced on adjoining sections up to the surface of the cortex. At the termination of the remaining experiments two fine wires were inserted into the postcentral gyrus through the electrode carrier, before removing the lucite chamber.

The wires were therefore in the same plane as the electrode tracks. They were inserted at either the upper or the lower margin of the trephine hole, and were left in situ during fixation. The block of the postcentral gyrus was cut parallel to the plane joining the two wires. Following this procedure most of the tracks appeared on a few adjoining sections.

With experience it was found that the most efficient method of finding the tracks was not to work systematically through the sections, but, from the photographs, to find on the sections a given vascular landmark. From this point each and every blood vessel on the surface of the gyrus was traced on successive sections in a medial or lateral direction—the branching of arteries and union of veins being noted—until one came close to the position of an electrode insertion marked on the first photograph. A detailed examination was then made of the relevant sections. An electrode track was only accepted as such if it conformed to all the following criteria:

(1) It could be traced continuously up to the surface of the cortex.
(2) It agreed precisely with the position marked on the photograph in relation to adjacent vessels and to the description of the insertion in the protocol.
(3) Its depth agreed approximately with that given in the protocol. The track was never quite as deep as the actual penetration as read from the electrode carrier dial, for the deepest portion of the track was not visible.

The tracks of 79 of the 99 electrode penetrations made in the 22 brains sectioned were found, and it may be stated with confidence that each of these is authentic. For the 20 tracks which were not found the precise sections, and the positions on these sections at which they should have been seen, were identified from the vascular pattern. These tracks were not found because the electrodes were not inserted sufficiently deeply into the brain. If the depth of the electrode penetration was less than 2.5 mm. no sign of the track can be found. After this fact was known all the electrodes were inserted to a minimum depth of 4 mm. It is reassuring, however, to know that the cortex suffered so little damage from the very gradually tapered electrode.

The shrinkage of the tissue due to fixation and histological preparation was estimated by the following method. The wires inserted at the end of the experiments to give the plane of section were a known distance apart. The distance between the tracks of these wires was measured on the sections. Comparison of these two figures in several brains gave a mean value of shrinkage of 26 per cent.

Photographs were taken of sections to show each track, one at 26 × and another at 52 ×. The line of the tracks was marked on the photographs. Where the tracks appeared on several successive sections the middle section of these was photogaphed and the actual angle of penetration determined by comparison of several sections. The correlation of electrical activity of neurons and the depths of the cellular layers proved, in these reconstructions, to be remarkably close.

RESULTS

Part I. The relation of functional properties of single cortical neurons to cortical cytoarchitecture

The method of single unit analysis provides a powerful tool for the study of the functional properties of the cells of the central nervous system. For neurons of the postcentral somatic sensory cortex we consider the following to be among the functional properties of interest and importance: (1) the form of the adequate peripheral stimulus which will cause the neuron to dis-

charge, i.e., its submodality type; (2) the location and size of the peripheral receptive field the stimulation of which will cause the neuron to discharge; (3) the security of the relation between the peripheral stimulus and the response of the cell, the *security of driving*; this latter is indicated by the unresponsive times of the system, by the frequency-following relation of cell response to peripheral stimulus, by the relation of the degree of the response to the intensity of the peripheral stimulus, and to the position of the stimulus within the peripheral receptive field; (4) the relation of the spontaneous and evoked activity of a neuron to that of its neighbors. Data concerning certain of these properties are presented below, are correlated with the morphology of the postcentral gyrus, and are used in the formulation of an hypothesis concerning the functional organization of this cortical area. Some of our findings relating to others of these properties appear in the two following papers (11, 12).

(1) *The general relation of the responses of cortical neurons to peripheral stimuli*

The first very obvious and uniform observation one makes when studying single neurons of the somatic cortex is that the large majority may be excited to action by mechanical stimulation of the body tissues. The figures of Table I, for example, show that even in the animal's anesthetized state 1436 of the 1648 neurons whose spontaneously occurring or evoked action potentials we have succeeded in isolating for study could be activated by peripheral stimulation.[3] Since the clear effect of a slight further deepening of the anesthetic state is to render securely driven neurons less so, and insecurely ones undrivable, it seems reasonable to conclude that in the waking state all, or nearly all, neurons of the somatic cortex may be responsive to peripheral stimuli. The possibility does exist, however, that there is a class of neurons of this cortical area whose elements are never spontaneously active under the conditions of these experiments, and which are unaffected by peripheral stimulation. If such a group exists it is at present beyond our means of investigation.

One of the most striking features of the activity of neurons of the postcentral gyrus is the difference in the occurrence and frequency of the spontaneous activity of neurons of area 3 on the one hand, and that of cells of areas 1 and 2, which occupy the exposed bank of the gyrus. So far as we are able to determine, this difference, which may be very great indeed, is not due to exposure of or damage to the gyral surface. Similarly, the very high level of activity of the cells of area 3 persists as the level of anesthesia is pushed to depths at

[3] Table I also indicates that unresponsiveness is commonly related to initial positivity of the electrical sign of the conducted impulses of cortical neurons, a fact determined, it is thought, by the spatial relation of the recording microelectrode tip to the active cell. The relation of mechanical damage, unresponsiveness, and initial positivity of electrical sign has been discussed in an earlier paper. (10)

TABLE I

Distribution of Submodality of Mechanoreception of All Cortical Neurons Isolated and Studied

		Total	Per Cent
Related to the skin		593	41
Quickly adapting	437		
Slowly adapting	153		
Undetermined	3		
Related to deep tissues		843	59
Joint	325		
Periosteum	204		
Fascia	314		
Total	1436		100
Units isolated but undrivable		212	
Initial sign positive	173		
Initial sign negative	39		

which that of cells of area 1 and 2 is very greatly reduced. Penetration of the microelectrode tip into area 3 is always signalled by a crescendo increase in the spontaneous activity; a whole host of neurons is spontaneously active, and individual ones at quite rapid rates. Actually the number of cells active is so great that isolation of the discharges of any one cell is more difficult here than in areas 1 and 2. A large majority of the cells of area 3 are activated by stimuli delivered to the skin (see Fig. 2). The security of the synaptic linkages of the pathway from the skin to the cells of area 3 is shown by the facts that, in comparison with the cutaneous neurons of areas 1 and 2, they follow more rapid rates of stimulation, they have shorter unresponsive times, and they are more resistant to depressing agents, such as anesthetics. Undoubtedly these properties of the neurons of area 3 reflect in large measure the extraordinary density of the thalamocortical projections from the ventral posterior thalamic group which terminate in this region of the somatic sensory cortex (3, 14, 22).

(2) *The submodalities of somatic sensibility*

The second uniform observation one makes upon postcentral neurons is that, while the great majority are sensitive to mechanical stimulation of the body tissues, a given neuron is not universally responsive to any and all forms and places of peripheral stimulation. On the contrary, cortical cells display very limited peripheral receptive fields (*vide infra*, and 12). They are selectively excited by stimulation of receptor organs within either the skin or the deep tissues of the body, but not, in our experience, by stimulation of both. At first glance this property might be regarded as a further topographic dis-

tribution of peripheral fields, in this case in depth in the body tissues. But those related to the skin and perhaps also those related to the fascia are so disposed to depict events properly classified as touch-pressure, and those activated from joint receptors to picture so exquisitely the angular positions of the limbs, and changes in those angles, that we are led to the conclusion that these two are separate submodalities of general somatic sensibility. Considerable supporting evidence for this idea will be presented below, and the conclusion fits with both clinical and psychophysical observations. We would like to emphasize that these two are thought of as *sub*-modalities of the general sense of mechanoreception, and are not at all equivalent to the classical modes of somatic sensibility: heat, cold; pain and mechanoreception.

This division of cortical neurons into these two groups, cutaneous and deep, is the key observation for much of that which is to follow. It is therefore requisite to examine the validity of such modality identifications before great significance is attached to this functional property. We believe that this identification can be made with considerable assurance, for the following reasons:

(a) Neurons driven from the receptors of the skin are not excited by stimulation of the deep tissues, after the elevation or removal of the skin bearing the receptive field.

(b) Neurons driven from receptors located in deep tissues are still driven by stimuli delivered to those deep tissues after the skin of the region has been removed. Direct dissection of the limbs allows the demonstration that their receptive fields are in fascia, periosteum, joint capsules, or deep connective tissue; we have not observed any within the muscle itself.

(c) Neurons driven from receptors located in the skin are responsive to electrical stimulation of the skin. Those related to deep tissues are not excited by electrical stimulation of the skin.

(d) We have not observed the sub-modality type of a cortical neuron to change, although we have repeatedly re-examined to determine the constancy of this property. This constancy has persisted under a variety of experimental conditions: change in depth of anesthesia, of blood pressure, of body temperature; it has persisted through episodes of spreading cortical depression, and through the progressive stages of cellular damage. Several neurons have displayed this mode stability for periods of observations longer than four hours, and we have studied 101 neurons for periods longer than thirty minutes without observing a modality shift. The point we wish to emphasize is that while it is possible that excitatory interaction of the two types of input upon the same single cortical cell may occur, we have not observed it under our experimental conditions. It may, of course, be a usual event under other experimental conditions, in the normal waking state, or at some further state of elaboration of cortical activity than that observed in these experiments.

(e) We have observed a cross-modality inhibitory effect, which is described in the following paper (11). Cortical inhibition is so tenuous an event, and so sensitive to the experimental conditions, that it seems very unlikely that we would have observed it and failed to observe excitatory interaction, which, by all other measures of excitation of cortical cells by peripheral stimuli, should be served by connections the more resistant to experimental vagaries.

We conclude from these observations that the sub-modality property of a cortical cell of the postcentral gyrus is, under our experimental conditions, a constant functional attribute of that neuron. It can be determined with considerable accuracy, and is an important property of a cortical cell.

(3) *Distribution of cortical neurons between submodality classes*

The data of Table I indicate the distribution between these two general categories of all the neurons we have studied. Those sensitive only to cutaneous stimuli may be further subdivided into those adapting quickly and those continuing to discharge at more or less steady rates, when steady skin pressure is applied. In general the quickly adapting neurons are sensitive to hair movement, and the slowly adapting one to light pressure upon the skin surface. This relation is not exclusive, however, for cortical cells of each type have been observed whose peripheral receptive fields (RFP's) lay exclusively in hairless portions of the body surface). Similarly, the neurons driven by stimulation of deep tissues may be further divided into those related to the joint capsules, to the periosteum, and to the fascia. It is the first of these which clearly plays a role in the general position sense and kinesthesis.

(4) *Location of neurons in areas 1 and 2 in relation to depth*

The chart to the left of Figure 1 indicates the distribution in depth below the cortical surface of all cells of areas 1 and 2 which were isolated, identified by submodality and studied. The distribution peak at a depth of about 900–1000 μ is, we believe, the result of at least two variables of the experiment. Firstly, it is much easier to isolate neurons in layers III and IV of the cortex than in more superficial layers, and only isolated neurons entered the population. Active, drivable cells may, in fact, be encountered at all levels from 100 μ to about 2000 μ during a penetration into the exposed surface of the postcentral gyrus. Secondly, when the anesthetic level deepens even slightly, as occurred frequently in our experiments, active and drivable cells are more commonly encountered in the middle layers of the cortex, which fact contributes also to the peaked distribution shown. The neurons of area 3 were reached by slanting micro-electrode penetrations made more or less parallel to the plane of the central sulcus, which makes a precise determination of their true depths below the cortical surface difficult. Nevertheless, all of our observations on this area indicate a depth distribution from the buried face

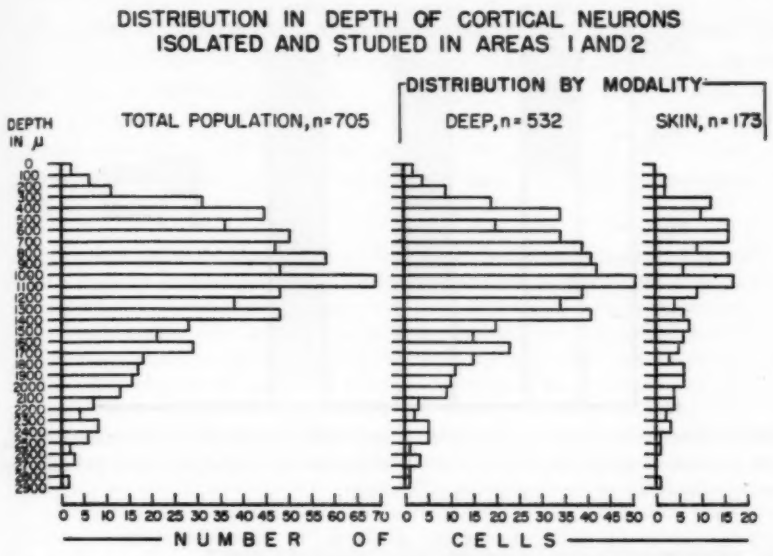

FIG. 1. Distribution of cortical neurons in depth below the pial surface of areas 1 and 2 of the postcentral gyrus of the monkey. Charts show that neurons of each submodality class occur in each of the cellular layers. The three distributions shown do not differ significantly from each other. Reasons for believing the peaked distributions, with maxima in the middle layers, to be due at least in part to experimental conditions are given in the text.

of the posterior bank of the gyrus which is quite similar to that shown for areas 1 and 2.

The two charts to the right of Figure 1 indicate that neurons of each submodality show similar distributions in depth. The two distributions are not significantly different from each other, or from the combined distribution.

(5) *Distribution of submodalities of mechanoreceptor sensibility in relation to cytoarchitectonic gradients*

It has been a matter of considerable interest to us to determine what differences, if any, exist between the functional properties of cells of the different cytoarchitectural areas of the postcentral gyrus. The chart of Figure 2 indicates a remarkable differential distribution of submodality types. The great majority of the cells of area 3 were activated by cutaneous stimuli (66 per cent), whereas in area 1 only 30 per cent were sensitive to such superficial stimuli. In area 2, on the other hand, about 90 per cent of the 191 neurons studied fell into the "deep" classification. This trend is indicated in greater detail by the chart of Figure 3, which shows that there are no abrupt transitions, but a gradual shift from a preponderance of cutaneous elements to one

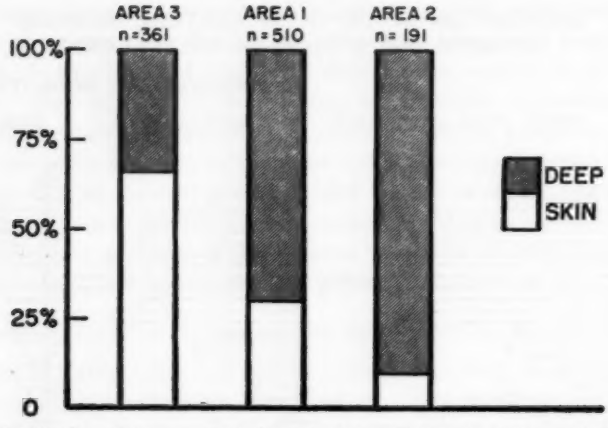

FIG. 2. Distribution of the neurons isolated and studied in each of the architectonic areas of the postcentral gyrus, divided between those activated by stimulation of the skin and those by stimulation of the deep tissues of the body.

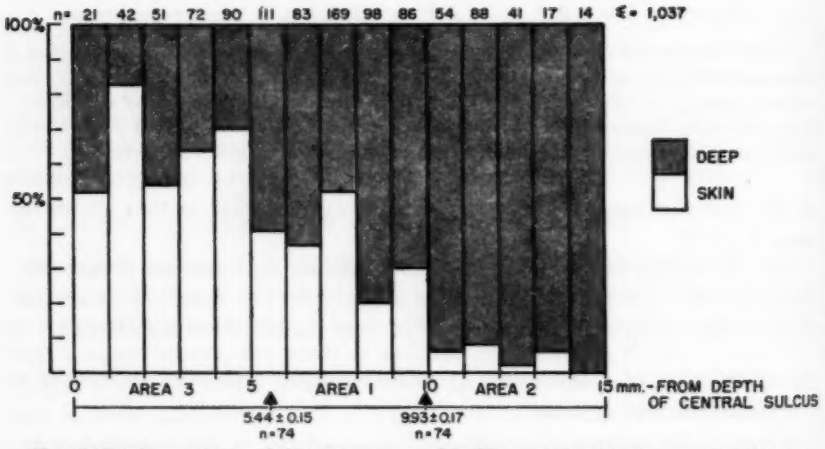

FIG. 3. Further analysis of the data presented in Figure 2. Population now divided into classes by mm. distance measured from the location of the cell to the depth of the central sulcus. It could not be made for 25 of the cells included in the totals for Figure 2. The shift from a preponderance of neurons of the deep class to one of those of the cutaneous class as position is translated forward across the postcentral gyrus occurs as a gradient, and not in abrupt steps. First mm. class, at depth of central sulcus, includes neurons of the transitional cortex, area 3-a.

of deep, from before backwards across the three cytoarchitectural areas of the postcentral gyrus.

There is one exception to this statement. In the first mm. behind the line marking the depth of the central sulcus there is an abrupt increase in the

proportion of deep units encountered, a sharp change from the trend immediately posteriorly. At most mediolateral levels of the postcentral gyrus this first mm. is made up either in part or wholly of the cortex of area 3a. It is our impression that this region of transition between the koniocortex of the postcentral gyrus and the motor cortex of the precentral does in fact receive a heavy projection from deep tissues. The data are so far too meagre to prove this statement.

The data shown in Figure 3 were obtained in the following manner. The track in the brain made by each microelectrode penetration was first sought in the serial sections (see methods). Seventy nine tracks of the ninety nine penetrations made in the brains of the first 22 animals studied were located, unequivocally. An enlarged print of the photomicrograph of each section containing a microelectrode track was made. The location of each unit isolated was marked on the print, and projected normally to the cortical surface. The distance of this projected point from the depth of the central sulcus was measured along the pia-arachnoid surface. The neurons were then grouped by classes at 1 mm. intervals of this measurement, the submodality type of each neuron was obtained from the protocols, and these data plotted as shown in Figure 3.

On the enlarged print of each photomicrograph the divisions between the three cytoarchitectural areas were indicated, and their distances from the depth of the central sulcus measured. The resulting data are shown by the mean figures at the bottom of the chart of Figure 3. They indicate that these divisions between cytoarchitectural areas can be made with remarkable uniformity from section to section and from brain to brain, if, as was the case here, the sections are taken from a rather limited mediolateral region of the postcentral gyrus.

Part II. The columnar organization of postcentral somatic sensory cortex

(1) *The submodality properties of neurons encountered successively in cortical penetrations*

In an earlier investigation of the cat's somatic sensory cortex (9) it was found that in the large majority of penetrations of the cortex the neurons encountered were sensitive to stimulation of either the skin or the deep tissues, and that elements of the two classes were not indiscriminately mixed. This was true if the electrode penetrated the cortex along a line more or less parallel to its true vertical axis, and thus normal to its surface. This evidence gave support to the idea that the elementary functional unit of the cortex is a vertical column of cells, and that those vertical columns related to various submodalities of sensation are arranged in a mosaic which in sum makes up the cortical projection pattern. A major aim of the present study was to test this hypothesis in the monkey postcentral gyrus under carefully controlled conditions of sub-modality identification, and with a precise histological demonstration of the site of entry, direction of travel, and depth of penetration for each electrode insertion.

The monkey postcentral gyrus is ideally situated to test this idea. The

TABLE II

Classification by Submodality of Penetrations Identified Histologically

Area	Pure Deep	Pure Cutaneous	Mixed	Total
1	22	8	20	50
2	18	2	3	23
3	2	6	29	37
(Totals	42	16	52	110*

* This number exceeds the total number of penetrations identified (73) because 37 electrode passages traversed both areas 2 and 3, and these enter both populations.

cortex of areas 1 and 2 of its exposed surface is relatively flat, and the cranial chamber can be arranged so that the electrode penetrates very nearly normal to the cortical surface. This is especially true in that flat portion of the gyrus which lies between 1 and 4 mm. lateral to the lateral end of the postcentral dimple. Anteriorly towards the bank of the central sulcus, the vertical axis begins to roll, and finally makes a rotation of about 90 degrees, coming finally in area 3 to be perpendicular to the buried posterior bank of the sulcus. This arrangement provides an opportunity for a large number of control observations. Electrode penetrations anteriorly in area 1 will be made at a variety of angles to the vertical axis of the cortex, that angle depending upon the exact antero-posterior position of the penetration. In passages into area 3 the electrode tip will, of course, be moving across that cortex parallel to its surface.

The raw data obtained are presented in Table II. In area 2, 87 per cent of penetrations were modality pure, in area 1 60 per cent were modality pure, while in area 3 only 22 per cent were so. Many of the penetrations into area 3 were, in fact, very incomplete (see Figs. 4, 5, 6, and 7) and it is our belief that had they extended to the full depths of the sulcus the per cent of area 3 penetrations showing modality purity would have been very small indeed. It is clear from these results that the predictions of the present hypothesis are confirmed.

Some interest attaches to the data which are not included in Table II, i.e. those penetrations whose tracks were not located in the serial sections. Most of these were made into the exposed surface of the gyrus, through the full thickness of the cortex (about 2000 μ). Of these 20, eleven were pure deep, 5 were pure skin, and 4 were mixed. This figure of 80 per cent modality purity fits with observations made in penetrations of area 2 and into the posterior part of area 1, which are included in Table II. It indicates that the tabulation of only those penetrations whose tracks were found in the sections has introduced no bias.

Very stringent criteria have been used in making the classifications indicated in Table II. In all, 79 tracks of electrode penetrations were found and traced in the 22 brains sectioned. In six of these penetrations technical difficulties prevented the making of observations, and

only the remaining 73 are considered in Table II. For a penetration to enter the classification as "submodality pure" it was required that: (a) all neurons whose discharges were isolated for study during the penetration belong to the same class, (b) neurons whose activity was observed in a multiunit record belong to the same class. The inclusion of data obtained when the action potentials of two, three or four neurons are observed in the record permits a tremendous increase in the ratio of sample observed to the total number of neurons at least potentially observable during a penetration, and (c) all data observed during electrode withdrawal fit that obtained on penetration.

We now wish to present the data obtained in a more detailed fashion. The bulk of that data is presented on the tracings of typical saggital sections of the postcentral gyrus, at different mediolateral levels.

For these illustrations the electrode tracks within a mm. or so of each other in the mediolateral dimension are shown as if occurring in the same single section. The typical section was selected by superimposing the outline tracings of the actual sections containing each of the electrode tracks to be illustrated. For example, 36 electrode penetrations were made in a region between 1 and 3 mm. lateral to the lateral end of the postcentral dimple, at a great variety of antero-posterior positions. The typical section chosen to represent this region and upon which to show the data from some of these penetrations (Figs. 5 and 6) is taken from a mediolateral position indicated by A in the inset drawing of Figure 5. This section matched almost exactly the mean of the superimposed drawings of all the sections. The section used for Figure 7 is at the mediolateral level marked B on the inset drawing of that figure and that of Figure 8 at the level marked C on its inset drawing. The angle of entry, distance of entry from the central sulcus, and the depth of travel were determined for each penetration from the study of the serial sections, the figures corrected for shrinkage, and the data transposed to the common drawing; they are indicated by lines passing down through the cortex. Where these lines are dashed no neural activity was observed; where they are continuous, active and drivable neurons were observed. Crosshatched areas to the right indicate that a multi-unit record evoked by stimulation of deep tissues was observed. Each horizontal line to the right indicates that at that level the isolated discharges of a single neuron driven from deep tissues were observed. Cross-hatched areas and horizontal lines to the left indicate, respectively, a multiunit record and isolated units related to the skin.

The drawing of Figure 4 illustrates data obtained in three penetrations made in experiment PPC 12. M12-P3 and M12-P5 were made at the same mediolateral level, and their tracks were found in the same histological section. M12-P4 was located in a section 400 μ farther laterally. All three are shown here on the tracing of the section containing the tracks of the first two penetrations; the section is very close to but not exactly at the mediolateral level marked A on the inset drawing of Figure 4. It is clear from the reconstructions that all three tracks enter the cortex almost parallel to one another and at an angle to the true vertical axis of the cortex which is very close to zero. All three crossed a small extent of white matter, and then penetrated for from 1.5 to 2.0 mm. into area 3. The penetrations into area 3 move diagonally across the true axis of the cortex, and pass in a long slant from deep to more superficial layers.

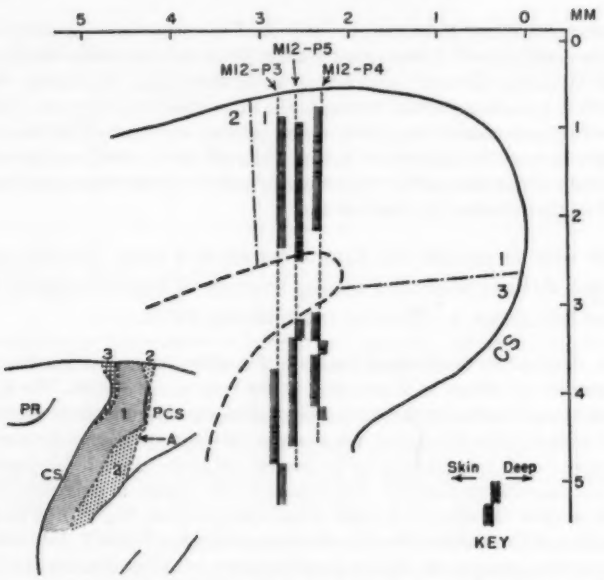

FIG. 4. Outline tracing of a saggital section of postcentral gyrus of monkey 12, taken at mediolateral level marked A on inset drawing. Tracks of M12-P3 and M12-P4 were found in this section, that of M-12-P5 a few sections farther laterally. All these are reconstructed as if occurring on this same single section. They are nearly parallel to each other and to the vertical axis of the cortex of area 1. During their traverses across area 1 each encountered neurons of the same single sub-modal class. Cross hatching and horizontal lines to the right indicate, respectively, a multiunit record observed and an isolated neuron studied, driven from deep tissues; similar symbols to the left indicate that stimulation of the skin evoked the neural activity observed.

All three penetrations passed across white matter, and the dashed lines indicate that no neural activity was observed during that portion of each penetration. Within area 3, each electrode encountered alternating blocks of tissue devoted to first one and then to the other of the sub-modal classes. Cells of the two groups are not randomly intermingled.

These three penetrations through area 1 are parallel to its vertical columns of cells. They were "modality pure". In M12-P4 each of the ten neurons isolated was activated by light tactile stimulation of the skin over the proximal phalanx of the fingers and the dorsal and preaxial hand. All multiunit observations confirmed these modality identifications, as well as the restricted and only slightly shifting peripheral receptive fields of the units encountered (see Fig. 10). In M12-P5 eighteen of the twenty neurons isolated were activated by rotation of the elbow joint—the remaining two by rotation of the humerus on the shoulder. All of the multiunit observations again confirmed the modality purity in this penetration. In M12-P3 each of the ten neurons isolated and studied was driven by mechanical stimulation of the distal end

of the radius and the bones of the wrist—the responsible receptors were thought to be in the periosteum. Again all the multiunit observations agreed with those made upon isolated units.

All three of these electrode penetrations passed across portions of the white matter, and no neural activity was observed in that region (dashed lines). In the reconstruction there is a fairly good fit between the disappearance and reappearance of neural activity and the movement of the electrode out of the cellular layers of area 1 above and its entry into cellular portions of area 3 below. In each case when the electrode entered area 3 modality purity was lost, the modality identifications successively alternating—in M12-P4 changing four times in a distance of 1.2 mm. An exceedingly important fact is evident here, and in the following illustrations, for *the alternation of submodality types of the neurons observed occurs between blocks or segments of cortical tissue; neurons of the two subtypes are not randomly intermingled.*

Nearly half of our penetrations were made in the region 1 to 3 mm lateral to the lateral end of the postcentral dimple. Of the 36 available, 3 are shown in Figure 4, and 16 are shown on the charts of Figures 5 and 6. The selection was determined only by convenience for illustration. Seven of the 16 penetrations made in the mediolateral region marked B and 6 of the 14 made in the region marked C are shown in Figures 7 and 8, respectively. These data will be considered by cytoarchitectonic areas.

Area 2.—The data from 14 penetrations in area 2 are shown in four figures (5, 6, 7 and 8). In 12 of these all the neurons were activated from receptors in deep tissues, in two all were responsive to skin stimulation. The location of area 2 is most advantageous for orientation of the recording electrode. The mean angle between the electrode track and the true vertical axis of the cortex in these 14 pure penetrations was about 5 degrees.

Area 1.—The data from 15 penetrations made into area 1 are shown in Figures 5, 6, 7 and 8. It will be seen there that as the site penetrated was shifted forward, and as the angle of entry was changed in some experiments to permit access to area 3, the degree of *en bloc* modality change increased remarkably. This increase in the degree of mixing is, we believe, due to two factors. Firstly, the angle of entry required to allow penetration into area 3 is a considerable one relative to the true vertical axis of the cortex of area 1. Secondly, that axis begins to roll as one approaches the central sulcus. Thus the *en bloc* modality changes encountered in such angular penetrations provide in these control observations a confirmation of the columnar hypothesis.

In one experiment the cranial chamber was arranged so that the angle of entry was parallel to the true vertical axis of area 1 very close to the bank of the central sulcus. A reconstruction of one of the penetrations made in this experiment (M9-P3) is shown in Figure 6. All the neurons observed here were driven from the skin of the controlateral hand (for fields, see Fig. 9).

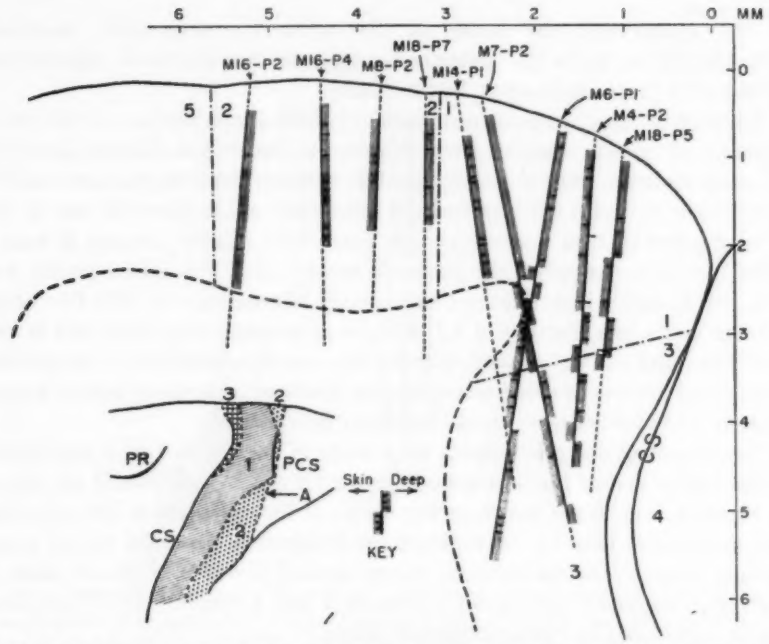

FIG. 5. Reconstruction of 9 of the 38 penetrations made within 1 mm. of the mediolateral level marked A on the inset drawing. Reconstructed tracks are shown as if occurring on an outline drawing which represents the mean contour of the 38 sections containing tracks of these penetrations. The following points are to be noted: modality purity in area 2 and posterior area 1; increasing mixing of modalities encountered as the site of penetration is moved forward to lip of central sulcus, where the vertical axis of the cortex is rolling; block alternation of cells related to one or another subgroup as electrodes penetrate area 3. Symbols as for Figure 4. Three "randomly mixed" neurons indicated.

Area 3.—No one of the penetrations has passed completely across area 3. The segmental mixing of submodality types of neurons encountered in these penetrations is obvious. In all of our data there are only 18 neurons which were indiscriminately placed—each appeared from the midst of a multiunit record of neurons of the other sub-modality. Six of these are indicated in Figures 5, 6, and 7. In this group of neurons we have frequently observed the cross-mode inhibition described in the following paper (11). We would like to emphasize that the common observation is an alternation of sub-modality types by segments of tissue, without random mixing. It leads to the conclusion, we believe, that the cells of area 3 are arranged in columns vertical to its true surface, columns of cells that are related to only one form of mechanoreceptive sensibility, and which make up a unit for function. An additional point of importance should be made. That is that the electrode tip *may* pass for dis-

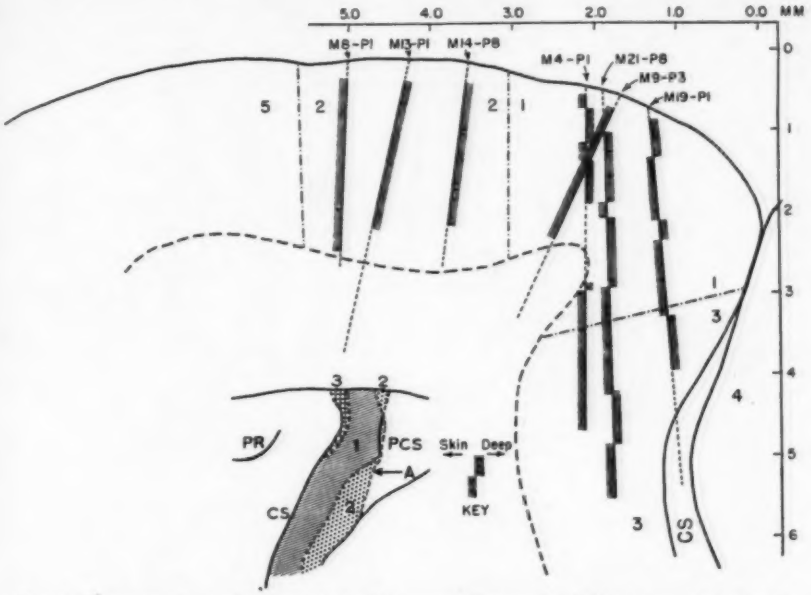

FIG. 6. Reconstruction of 7 more of the 38 penetrations made within 1 mm. of the mediolateral level marked A on the inset drawing. Reconstructed tracks are shown as if occurring on an outline drawing which represents the mean contour of the 38 sections containing these electrode tracks. Symbols as for Figure 4; comments as for Figure 5.

tances as great as 1 mm. across area 3, without encountering a change in the submodality represented (for example M4-P1 and M21-P8 of Fig. 5; M6-P1 of Fig. 6; M20-P1 of Fig. 8). We shall return to this matter below.

(2) *The Receptive fields of neurons isolated in depth along the transverse axis of the cortex.*

One result of earlier single unit studies of the cat somatic sensory cortex (9) was that the topographic pattern of representation of the body form on the cortical surface extends unchanged throughout the cellular layers of the cortex. Neurons successively encountered in vertical electrode penetrations are activated from peripheral receptive fields confined rather strictly to the same local region of the body, and this observation fits well with the idea of the columnar organization of the cortex. That this fact is true also for the cortex of the postcentral gyrus is supported by all of our data, and is illustrated in Figures 9 and 10.

The illustrations of Figures 9 and 10 were made in the following way. During the experiment receptive fields were mapped by observing the relation between light mechanical stim-

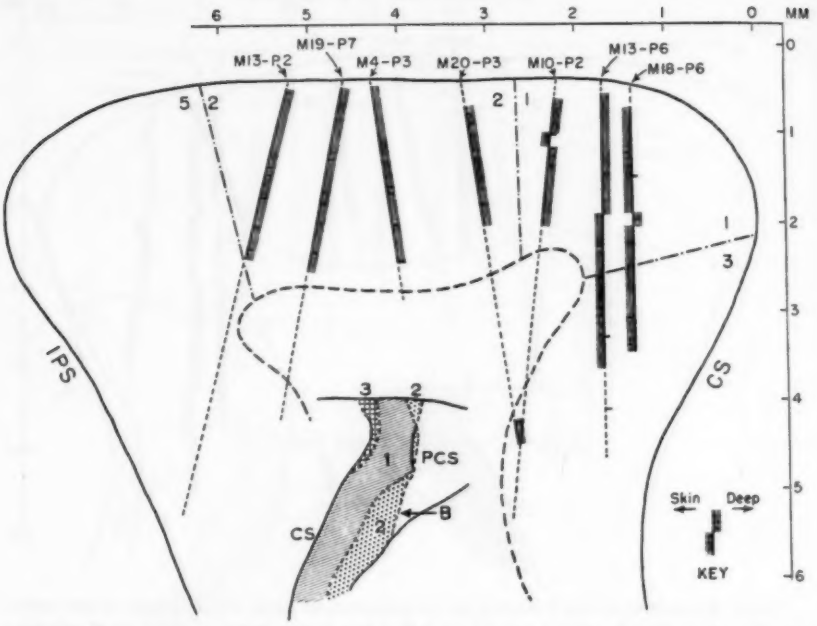

FIG. 7. Reconstruction of 7 tracks of electrode penetrations made at or very close to the mediolateral level marked B on the inset drawing. Tracks are shown as if all occurring on an outline drawing which represents the mean contour of the seven actual sections containing these seven electrode tracks. Symbols and comments as for Figures 4 and 5.

ulation delivered to the periphery and the response of the neuron under study. These fields were marked on the skin with washable ink and the body part photographed. Later these photographs were projected to natural size and drawn. Superimposition of the tracings for all of the units, in a given penetration, whose fields were determined resulted in drawings of the type shown in Figures 9 and 10.

The location of the electrode tracks of the penetrations illustrated in Figures 9 and 10 are to be found as follows: of M3-P6 in Figure 7; of M9-P3 in Figure 5; of M12-P4 in Figure 4; of M4-P1 in Figure 5; of M6-P1 in Figure 6; and of M13-P6 in Figure 7. Tracings of sections with course of electrode tracks and indications of the data obtained are shown in Figures 9 and 10 for M19-P10, M8-P4; and M8-P6. The data obtained are indicated for M17-P1, but the track could not be found in the sections. It clearly passed through area 1 at an angle, then crossed the intervening white matter, and penetrated into area 3.

It is clear from the sections and from Figure 5 that M9-P3 passed into area 1 almost exactly parallel to the vertical axis of the cortex. In its course the many neurons encountered had receptive fields located on the hypothenar eminence, volar wrist, or postaxial side of the contralateral hand. Seven of these fields are shown in Figure 9. A more perfect superimposition of fields was observed in M15-P3, for here all seven of the neurons isolated had fields

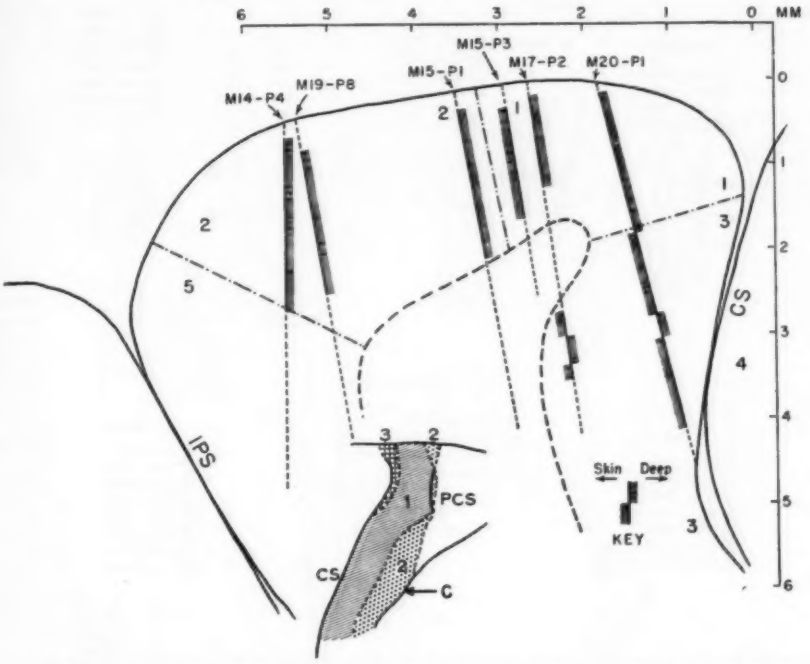

FIG. 8. Reconstruction of the tracks of six electrode penetrations made at or close to the mediolateral level of the postcentral gyrus marked C on the inset drawing. The six are shown as if occurring in the same single section, which represents the mean contour of the six actual sections in which the six electrode tracks were found. Symbols and comments similar to those for Figures 4 and 5.

limited to the hypothenar eminence of the contralateral hand. A restriction of the somewhat larger fields of the neurons encountered in M19-P10 to the inner side of the contralateral thigh is shown also in Figure 9.

A comparison of the skin fields encountered in area 1 with those of area 3 below, in the same electrode passage, is available for many penetrations, two of which are illustrated in Figure 9. In M12-P4 (course of track shown in Figure 4) the neurons of area 1 had fields on the dorsum of the hand and fingers, while those encountered in area 3 had fields on the dorsal or ventral surfaces of wrist and lower forearm. In M3-P6 the seven fields mapped for neurons of area 1 were limited to the palm of the hand, while those of area 3 (only 1 is shown) were on the dorsal surfaces of the fingers. A third example is shown in Figure 10. The fields of neurons encountered in area 1 in penetration M17-P1 very nearly superimpose upon those of neurons studied in area 3 during the same penetration. In general it can be said that in such penetra-

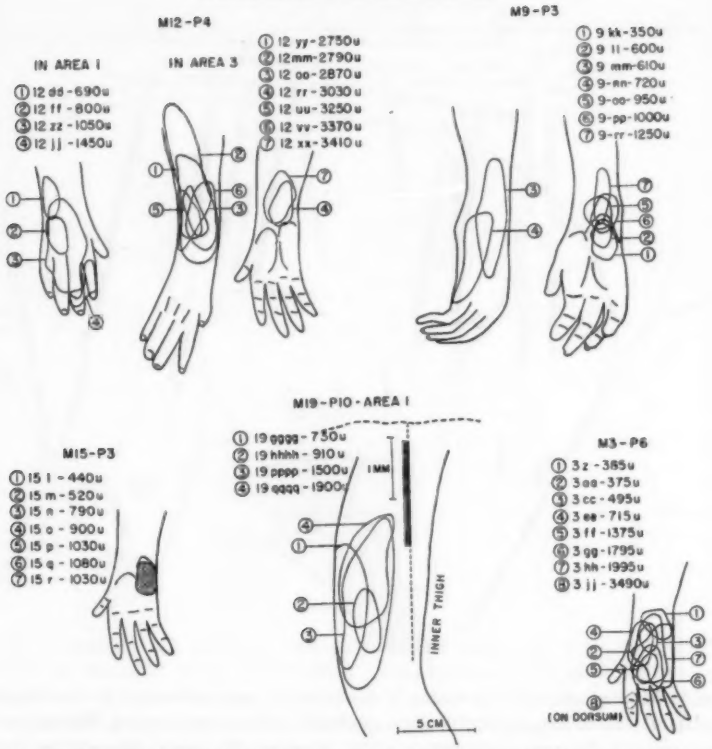

FIG. 9. Drawings of the peripheral receptive fields of neurons of the cutaneous subclass encountered in five penetrations of the postcentral gyrus. Reconstructed track of M19-P10 shown, those of the other four shown in previous figures. Note near superimposition of fields, which was nearly complete for the fields of the seven neurons studied in M15-P3. For M12-P4 note that fields for cells of area 1 differed very little in position from those for neurons of area 3 which were studied in the same penetration. In this and Figure 10 the centimeter scale refers to drawing of parts of the body, millimeter scale to depth below the cortical surface for the reconstructed electrode track shown.

tions the fields encountered below in area 3 will be very close to if not similar in position to those encountered above in area 1, in the same electrode track. The fields do not move from proximal to distal along the limb, to any significant extent, as the electrode moves deeper into area 3.

The "receptive fields" of neurons encountered in "pure deep" penetrations are just as restricted in peripheral location as are those just illustrated for cutaneous ones, a fact documented in Table III. The receptive fields of all the cells observed in a penetration may be limited to a single joint (M13-P4, M13-P3, M4-P3), or to two adjacent joints, with sudden shift from one to the other (M12-P5, shift from elbow to shoulder at depth of 860 μ). During a

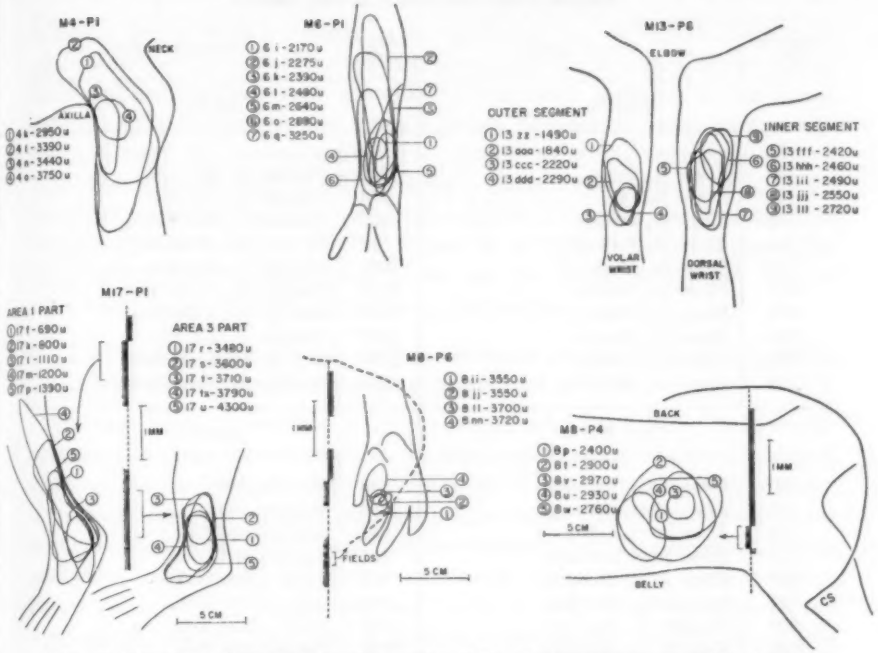

FIG. 10. Drawings of the peripheral receptive fields of neurons encountered in five penetrations of the cortex of the monkey postcentral gyrus. Reconstructions of penetrations M8-P4 and M8-P6 shown in the figure, track of M17-P1 not found in the sections; data shown. Reconstructions of M4-P1, M6-P1 and M13-P6 shown in earlier figures.

penetration the receptive fields may shift from a joint or periosteal location to adjacent deep fascia (M20-P3, M16-P2), or *vice versa*, but this has been an exceptional event.

(3) *The receptive fields of neurons isolated in penetrations made at angles to the vertical axis of the cortex of area 3.*

It has been stated above that the electrode may move for considerable distances across area 3 without submodality change for the units encountered,[4] and the remarkable fact is that on many such occasions the receptive fields of the neurons observed during the long traverse do not move progressively. Examples are given by M4-P1 and M6-P1 of Figure 10. In each case the receptive fields of cells distributed over nearly 1 mm. of brain tissue were confined fairly well to the same peripheral region. Occasionally the field shifted

[4] This is true when the slant is directed in the antero-posterior direction, as it is when vertically oriented penetrations pass down parallel to the posterior bank of the central sulcus. If the penetration has a medio-lateral slant, however, the receptive fields encountered will change very rapidly and continuously.

TABLE III

Receptive Fields of Neurons of the Deep Subclass

Depth of Unit	Driving Stimulus
M13-P3	
μ	
390	Elbow Jt., Sup.
420	Elbow Jt., Sup.
490	Elbow Jt., Flexion
600	Elbow Jt., Flexion
600	Elbow Jt., Flexion
670	Elbow Jt., Flexion
770	Elbow Jt., Flexion
770	Elbow Jt., Flexion
950	Elbow Jt., Flexion
1380	Elbow Jt., Extension
M12-P5	
380	Elbow Joint, Extension
460	Elbow Joint, Extension
720	Elbow Joint, Extension
750	Elbow Joint, Extension
860	Elbow Joint, Extension
860	Shoulder Jt., Ext. Rot.
900	Shoulder Jt., Ext. Rot.
1020	Shoulder Jt., Extension
1060	Shoulder Jt., Extension
1110	Shoulder Jt., Flexion
1140	Shoulder Jt., Extension
1250	Shoulder Jt., Extension
1375	Shoulder Jt., Extension
1500	Shoulder Jt., Extension
1500	Shoulder Jt., Extension
1550	Shoulder Jt., Extension
1680	Shoulder Jt., Extension
1750	Shoulder Jt., Flexion
1750	Shoulder Jt., Flexion
1830	Shoulder Jt., Flexion
1830	Shoulder Jt., Ext. Rot.
M16-P2	
830	Periosteum, Humerus
830	Periosteum, Humerus
990	Periosteum, Humerus
1020	Periosteum, Humerus
1020	Periosteum, Humerus
1320	Periosteum, Humerus
1345	Periosteum, Humerus
1470	Fascia, Upper Arm
1740	Fascia, Upper Arm
2000	Fascia, Upper Arm

Depth of Unit	Driving Stimulus
M4-P3	
μ	
730	Shoulder Jt., Int. Rot.
750	Shoulder Jt., Int. Rot.
850	Shoulder Jt., Int. Rot.
960	Shoulder Jt., Abduction
1000	Shoulder Jt., Int. Rot.
1110	Shoulder Jt., Protraction
1230	Shoulder Jt., Abduction
1270	Shoulder Jt., Int. Rot.
1650	Shoulder Jt., Abduction
2100	Shoulder Jt., Retraction
M20-P3	
390	Shoulder, Pro.
490	Shoulder, Abd.
810	Fascia, Chest Wall
815	Fascia, Chest Wall
915	Fascia, Chest Wall
940	Fascia, Chest Wall
990	Fascia, Chest Wall
1020	Fascia, Chest Wall
1040	Fascia, Chest Wall
1110	Fascia, Chest Wall
1110	Fascia, Chest Wall
1180	Fascia, Chest Wall
1440	Fascia, Chest Wall
M13-P4	
790	Hip Joint, Int. Rot.
900	Hip Joint, Int. Rot.
980	Hip Joint, Int. Rot.
1030	Hip Joint, Int. Rot.
1030	Hip Joint, Int. Rot.
1110	Hip Joint, Int. Rot.
1220	Hip Joint, Int. Rot.
1220	Hip Joint, Int. Rot.
1250	Hip Joint, Int. Rot.
1250	Hip Joint, Int. Rot.
1470	Hip Joint, Flexion
1630	Hip Joint, Abduct.
1630	Hip Joint, Abduct.

suddenly during such a traverse; an example is given by M13-P6 of Figure 10, in which, in the midst of a 1.6 mm. movement across area 3, the receptive fields suddenly shifted from the volar, for the outer segment, to the dorsal surface of the forearm for the inner segment. Occasionally very narrow segments of tissue containing neurons activated from the skin are crossed in area 3. In these cases the receptive fields of the neurons of the segment are similarly restricted to nearly the same peripheral location. Examples are given by M8-P4 and M8-P6 of Figure 10. The former is of particular interest from the topographic point of view, for it shows that the thorax and trunk may be represented in the very depths of the central sulcus.

(4) *The receptive fields of single cortical neurons in relation to the overall pattern of projection of the body form upon the postcentral gyrus*

We have made an analysis of the peripheral receptive fields of neurons encountered in penetrations made at the three mediolateral levels A, B, and C indicated on the inset drawings of Figures 6, 7, and 8. At each level the observations were upon populations extending across the full antero-posterior extent of the gyrus. We do not intend to present these data in detail, for an analysis of topography has not been a major object of the present study. Nevertheless, one conclusion from our studies is clear. That is, that even the most apical portion of the hand or the foot is represented in a band of cortical tissue extending across the entire antero-posterior extent of the postcentral gyrus. This is true also for the more proximal portions of the body, such as the trunk and the regions of the shoulder and pelvic girdles. Thus in our data the representation pattern resembles closely that described by Woolsey, Marshall and Bard (27) as a "dermatomal" or segmental projection. It is difficult to fit our observations with the idea that the cortical projection pattern is a distorted figure of the animal in which proximal parts are represented posteriorly and the apices of the limbs and face extend forward to the depths of the central sulcus. It should be emphasized, however, that studies of single neurons, even when based upon the reconstruction of large populations, cannot be decisive in this matter. The two ideas—of a segmental *versus* a distorted body form representation are not mutually incompatible. This problem becomes of interest and importance in relation to the sharp gradient of submodality representation across the postcentral gyrus in an antero-posterior direction, described in an earlier section.

DISCUSSION

1. The validity of submodality identification

The most obvious difference between neurons of the postcentral gyrus is that some may be activated only by stimulation of the skin, while others are

related only to receptors located in deep tissues. The functional properties of these two groups and their probable roles in tactile and deep sensibilities, respectively, are the subjects of the two papers which follow (11, 12). Here this distribution of the cells of the postcentral gyrus into two main classes is used in an analysis of the functional organization of that region of the cortex. For this reason considerable attention has been given to the validity with which the sub-mode type of a cortical cell can be determined in the experimental situation. Evidence that this determination can be made quickly and reliably has been given in some detail. It seems most likely that this remarkable specificity of a cortical neuron is determined by its anatomical connections, for the following reasons. (a) The sub-mode type is constant, and has not been observed to change with time, at least during observation periods lasting in some instances 3 or 4 hours. (b) It is independent of anesthetic level, and that level has been intentionally varied over a wide range, while a single neuron was under study. Other functional properties, however, are exceedingly sensitive to variations in depth of anesthesia (12). (c) Dissection has identified the location of the peripheral tissues bearing the receptors related to given neurons. (d) We have not observed excitatory interactions of cutaneous and deep stimuli upon single cortical cells, though an inhibitory interaction has been seen, and is described in the following paper (11). This absence of excitatory interaction is reported with the usual caution regarding negative observations, for under other experimental conditions it may occur.

We would like to emphasize that submodality identifications can best be made using *physiological stimuli*. It is now clear from anatomical studies that no peripheral nerve can be considered *a priori* to be "purely" cutaneous, for each may contain afferents from receptors in joint capsules, deep fascia, and other deep connective tissue. Evidence for this last statement is quoted in some detail in the following paper (11).

We conclude that identification of the type and location of a peripheral stimulus driving a postcentral neuron can be made reliably, and that this modal property is anatomically determined. This leads to the conclusion that modality, e.g., tactile or deep sensibility, is not a sensory datum signalled by the temporal patterns of activity of a cortical cell.

2. Distribution of postcentral cells of different sub-modes in relation to cytoarchitecture, topographical pattern, and thalamic projections

That the sub-modal property of a postcentral neuron is constant, depends upon its anatomical connections, and can be determined with some certainty are facts of considerable importance, for a differential distribution of neurons related to the skin and those excited by stimulation of deep tissues is the most striking difference we have observed to exist between the three cytoarchi-

tectural areas of the postcentral gyrus. The cells of area 2 are almost exclusively (90 per cent) of the deep class, but more anteriorly, in areas 1 and 3, progressively increasing proportions of cells are related to the skin. That this change is a gradual one without abrupt steps (see Fig. 3) fits well with the finding that the divisions between the three cytoarchitectural areas are regions of gradients of morphological change, rather than sharp lines (15). This is one functional expression of the very clear cytoarchitectural differences of the postcentral gyrus. We shall consider it in relation to the topographical pattern and the thalamic connections of the region.

In an extensive series of investigations detailed in papers and reviews, (23, 25), Woolsey and his colleagues have made a definitive study of the pattern of projection of the body form upon the somatic sensory cortex. The simple and continuous, though distorted, pattern found in rodents and carnivores (6, 26, 28) is present also in the postcentral region of semi-lissencephalic primates (1). In primates with more convoluted brains (macaque (27), spider monkey (2), chimpanzee (24), and apparently in man as well (13)), however, the simple continuity of the dorsal midline of the body, placed most posteriorly, with the apices of the limbs pointing anteriorly, has been broken. This disruption appears to have resulted from the extraordinary enlargement of the cortical areas for hand and foot. In these primates it has not yet proved possible to demonstrate by means of the evoked potential method the middorsal continuity of the representation pattern so characteristic of simpler forms. Whether this is a true departure in phylogeny or simply a tenuous cortical projection is an unsolved problem which, as Woolsey emphasizes (25), merits further study. These observations in the macaque are fully confirmed in our present study, which leads to the following conclusion. That is, that the afferent fibers of a dorsal root, comprising the full modality spectrum, are represented in a narrow antero-posterior band of cortex. Those afferents related to deep receptors of the segment are represented chiefly in area 2, those related to the skin chiefly in area 3, with area 1 representing a gradient between the two. This conclusion at once poses the question of what complex interactions may occur between areas 3, 1 and 2, for normal sensations are certainly the complex sum of the effects produced by afferent impulses in a wide variety of first order axons.

The differential distribution of neurons of different modal properties implies a differential projection of the ventral nuclear complex of the thalamus upon the postcentral gyrus. Such a differential projection has been described by Le Gros Clark and Powell (3). These workers studied the retrograde cellular degeneration and atrophy in this thalamic nucleus resulting from cortical lesions confined to either area 3, 1 or 2 of the postcentral gyrus of the macaque. Their conclusions are summarized as follows. Area 3 receives connec-

tions from cells located over the entire rostro-caudal extent of the nucleus, from most of the cells in its anterior half, and from at least 50 per cent of the cells in its caudal half. Area 1 receives the exclusive projection of some 30 per cent of the cells of the caudal half, and probably collaterals of axons which terminate mainly in area 3. Area 2 receives the exclusive projection of *no thalamic cells*, but is thought to receive the collaterals of thalamocortical axons ending mainly in areas 3 and 1. In the light of our present finding this suggests that the cells of area 2, 90 per cent of which are of the "deep" class, are activated by collaterals of axons impinging in the main upon the much smaller proportion of "deep" neurons of areas 1 and 3. This possibility has led us to a current study of the modality properties of thalamic cells and their distribution within the ventral posterior complex, a study which is presently under way.

We have no evidence as to where within the postcentral gyrus such modes as pain and temperature are represented. We can therefore offer no explanation for the very interesting findings of Russell (18) and Marshall (8), who studied the sensory defects persisting in men surviving injuries of the postcentral gyrus. The latter author observed that when the lesion was confined to the lateral surface of the gyrus (presumably areas 2 and 1) there was a loss of the discriminative forms of general somatic sensation on the contralateral side of the body. In so far as "discriminative forms" include position sense and kinesthesis, this observation fits well with our present findings (see the following paper, 11). Lesions confined to the regions of the central sulcus (presumably area 3) produced a loss of contralateral pain and temperature sensibilities, with retention of discriminative forms. In so far as pain and temperature sensibilities were referred to cutaneous structures, this also is compatible with our present findings. We can give no explanation for the additional and paradoxical finding of Marshall that lesions of the entire postcentral gyrus produced a loss of discriminative forms of somatic sensibility, but no alterations in the senses of pain and temperature.

3. The Columnar Organization of the Cortex of the Postcentral Gyrus

Earlier investigations of the somatic cortex of the cat produced evidence that the first-order functional unit of that region is made up of a vertically oriented column of cells extending across all the cellular layers of the cortex. The postcentral gyrus of the monkey has provided an ideal site to test this hypothesis. The cortex of the exposed bank of the gyrus is flat over a considerable distance, and the penetrating electrode can be oriented in such a way that it passes through the cortex parallel to its vertical axis, i.e. normal to the cortical surface. More anteriorly this axis of the cortex begins to roll and penetrations made here cross it at a succession of increasing angles, thus providing an intrinsic control of the hypothesis, as does also the fact that the

electrode crosses area 3 at or close to 90 degrees to its vertical axis. The findings concerning the modality purity of penetrations in the present study agree with the predictions of the hypothesis. Penetrations made into area 2 and the posterior part of area 1 are in the majority modality pure, and as the site penetrated is moved forward the percentage of "mixed" penetrations increases until, in area 3, the overwhelming percentage is mixed. (see Table II and Figs. 4 to 8).

Evidence obtained in these mixed penetrations is of great importance, for, as the figures indicate, the mixing of sub-modalities occurs by *blocks of tissue, and not randomly*. In our entire population of neurons studied (more than 1400) only 18 cells were observed in the midst of traverses in which cells of the other submodality were present—i.e., were randomly mixed. This block alternation of submodality observed during penetrations made at angles to the vertical axis of the cortex is the necessary control for, and corollary to, the first observation, i.e., that of penetrations made parallel to that axis, the overwhelming percentage are "modality pure".

The penetrations made across area 3 require some comment. Although in 78 per cent of these penetrations alternating blocks of tissue devoted first to neurons of one and then those of the other class were observed, it is still true that the electrode could be moved for considerable distances across area 3 without encountering a sub-modality change. In such long traverses, the electrode usually encounters neurons related to the skin. (Examples are: M12-P5 of Fig. 4; M4-P1 and M21-P8 of Fig. 6; M20-P1 of Fig. 8.) The fields of the neurons studied during such area 3 traverses are shown for several other penetrations in Figures 9 and 10. The important observation is, we believe, that the peripheral location of the fields of the successive neurons encountered is more or less constant, they do not gradually shift. This indicates that the electrode in such a traverse is passing across a considerable volume of tissue devoted to cutaneous receptors of a local part of the body. Consideration of the dwindling number of vertical columns devoted to deep receptors which is encountered in moving forward from area 2 across area 1 and into area 3 predicts that an electrode tip will, on chance, move for considerable distances in area 3 without encountering such a deep column. Such was the case.

The evidence presented in Figures 9 and 10 and in Table III, shows that cells encountered in vertical penetrations are related not only to the same submodality, but to nearly the same peripheral receptive field as well. This observation supports the idea of the vertical organization of the cortex and indicates also that the pattern of representation of the body form observed with surface recording extends throughout the depths of the cortex.

In previous studies on the cat (9) it was observed that the latencies of response of cells along the vertical axis of the cortex were not significantly different for the neuronal elements of different layers. All of our observations in the monkey fit also with the statement that

within a period of 2–4 msec. after the activation of the first cortical cells responding to the thalamocortical volley all the responding cells of the vertical column begin to discharge. Whether the latency differences within the 2–4 msec. period are significant and whether they indicate a sequential trans-synaptic activation of cells of the various layers is a matter on which we do not at present have completely telling data; it is a subject of continuing investigation. Some requirements for obtaining reliable data of this sort are clear. For each neuron encountered the receptive field must be mapped and that site within it whose stimulation produces responses of shortest latency determined. This "hottest spot" is frequently placed eccentrically in the field. It is equally clear that such data cannot be obtained by electrical stimulation of peripheral nerves or by local electrical stimulation of such synaptic relay stations of the system as the ventral posterior thalamic nucleus.

Thus all of our data obtained in the cat and the monkey agree with the columnar hypothesis. This idea fits also with the earlier anatomical studies of Lorente de No (7), and the later observations of Sperry (19) and Sperry, Minor and Myers (20). These latter authors have shown that cats with subpial vertical dicing of the cortex on a 1 mm grid are still capable of making very fine visual discriminations, and that monkeys in which the sensory and motor cortices have been treated similarly are still capable of what appeared to be normal motor activity. These observations of Sperry and his colleagues show very clearly that rather small vertical columns of cortex are capable of integrated activity of a rather complex order, which is independent of lateral intragriseal spread of activity.

We would like to emphasize that the vertical pattern of activity set up in the somatic sensory cortex by a peripheral stimulus is based upon an analysis of the *first* response of cortical cells to that stimulus. It should not be interpreted to mean that only this pattern of functional organization exists, for the cortical cells must certainly be grouped sequentially into various patterns subserving higher orders of cortical function, patterns of activity about which at present nothing is known.

SUMMARY

The modality and topographic properties of neurons of the postcentral gyrus have been determined by the method of single unit analysis, and these findings have been considered in the light of a re-study of the cytoarchitecture of this cortical region. Over 90 per cent of the neurons of area 2 are related to receptors of the deep tissues of the body, while the large majority of those of area 3 are activated only by cutaneous stimuli. This differential representation occurs as a gradient of change in the anteroposterior dimension of the postcentral gyrus, as do also the morphological variations of the region.

Data obtained during microelectrode penetrations of all three areas support the hypothesis that the first pattern of activation of cortical cells responding to a peripheral stimulus is that of a vertically oriented column of cells extending across all the cellular layers of the cortex. It is emphasized that this pat-

tern of activity pertains only to the first or early repetitive response of cortical cells, and does not preclude the sequential formation of other patterns which may be of quite different natures.

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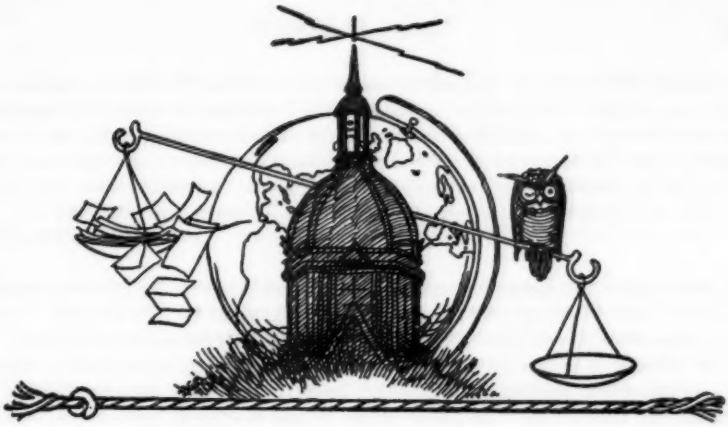

BOOK REVIEWS

An Atlas of Normal Radiographic Anatomy, 2nd edition. By ISADORE MESCHAN with the assistance of R. M. F. FARRER-MESCHAN. 759 pp., $16.00. W. B. Saunders Co., Philadelphia, Pa.

The new edition of this work contains amplified sections on cardiovascular radiology, neuroradiology and radiation protection. Many of the photographic reproductions of the original edition have been replaced or improved.

The nature of this book is accurately described by its title. It correlates the gross anatomy of the part of the body under consideration with the radiographic image. Being so fundamental, it is useful to technicians, medical students, house staff residents on various services and first-year residents in Radiology. The text is concise and well written and is accompanied by good illustrations. It does not attempt to treat radiographic pathology, but the important clinical indications for many of the radiographic procedures are listed.

DANIEL TORRANCE

Physiology of Cardiac Surgery. Hypothermia, Extracorporeal Circulation and Extracorporeal Cooling. By FRANK GOLLAN. 96 pp., $4.50. Charles C Thomas, Springfield, Ill.

This small monograph is an excellent compact presentation of pertinent physiological observations related to hypothermia and extracorporeal circulation. The material is presented in three sections, hypothermia, extracorporeal circulation, and combined hypothermia and extracorporeal circulation. The physiologic data are valuable for interpreting changes seen in clinical use of either method. Historical developments in both fields are briefly presented, and future lines of study are outlined.

The book should be useful to anyone actively working in intracardiac surgery. Its small volumes, however, makes it a too-advanced text to serve as an introductory text to intracardiac surgery. The literary style, with a liberal admixture of philosophy and historical quotations, is entertaining as well as informative.

FRANK C. SPENCER

Patient Care and Special Procedures in Xray Technology. By CAROL HOCKING VENNES AND JOHN C. WATSON. 203 pp., $5.75. The C. V. Mosby Co., St. Louis, Mo.

This little book presents in a concise and readable way a treatment of the clinical situa-

tions behind the various radiographic procedures. It is written for x-ray technicians and attempts to explain the techniques of proper patient handling in the x-ray department. Emphasis is always on considerate handling of the patient according to the nature of the clinical problem. There are special sections on bedside radiography, diagnostic examinations in general, neuroradiography, vascular radiography and operating room radiography. The x-ray technician will find a great deal of value and of interest in this book.

DANIEL TORRANCE

The Szondi Test in Diagnosis, Prognosis and Treatment. By LIPOT SZONDI, ULRICH MOSER AND MARVIN W. WEBB. 309 pp., $12.00. J. B. Lippincott Co., Philadelphia, Pa.

Ten years have elapsed since the publication of Deri's *Introduction to the Szondi Test*, a period of time in which the predictive validity of that instrument has been critically examined and found wanting. It is doubtful, however, that these negative findings have dampened the faith or the enthusiasm of the Szondi contingent one iota. As evidence, witness the volume under review.

The present work, intended as a basic text, consists of several more-or-less related chapters dealing with the various aspects of Szondi's theory. The content is by no means confined to test administration and interpretation, since it is the contention of the authors that ". . . . it is impossible to employ the Szondi test with any degree of accuracy outside the frame of reference in which it was developed." (p. vii). This is a very convenient statement, for it permits the authors to discount practically all of the negative results obtained by others with the test. It may be remembered in this connection that Deri, whose writings stimulated much of the research in this area, did not hold that it was necessary to embrace Szondi's theories in order to make meaningful use of the test materials.

Let it be said at this point that this book is hardly worthy of being dignified by a lengthy critical review. There are two very good reasons for this: In the first place, one would hardly know where to begin; and in the second place, practically all of the more pertinent criticisms have been made many times in the past. (As a beginning, the interested reader is referred to the section on The Szondi Test in *The Fourth Mental Measurements Yearbook* edited by O. K. Buros.) Still, perhaps a brief description of this work is in order.

Each chapter appears to have been written independently by an individual author, and several of the chapters are translations of articles that have appeared elsewhere. Approximately half of the book has been written by Webb, and it is to be assumed that the remaining chapters by Szondi, Moser, Boeszoermenyi, and Achtnich have been translated and edited by him as well. The content ranges from interpretations of test data in connection with specific cases to discussions of the techniques of schicksal analytic therapy. The prose is rather unusual and studded with unfamiliar terms as well as with familiar terms with unfamiliar meanings. This reviewer found several passages incomprehensible. With the exception of some very curious computations in chapters 6 and 7, statistical analyses in general and statistical tests of hypotheses in particular are definitely eschewed. While it must be admitted that many of the authors' assertions would hardly be amenable to confirmation or denial, they become, by the same token, scientifically meaningless. Still, questions of validity are begged at every turn. Deri's treatment of these problems might be considered rigorous by comparison.

In the last chapter by Szondi, the reader is told: "The most vigorous opponents of schicksal analysis we have found in the ranks of those psychiatrists and psychologists who are the descendants of psychically disordered ancestors or themselves become mentally disordered episodically." (p. 292).

So, you had better believe it.

JOHN W. SHAFFER

Mirage of Health. Utopias, Progress and Biological Change. By RENÉ DUBOS. Edited by RUTH NANDA ANSHEN. 236 pp., $4.00. Harper & Bros., New York, N. Y.

This fascinating book is one of a series, entitled *World Perspectives*, whose purpose is "to reveal basic new trends in modern civilization, to interpret the creative forces at work today . . . . and to contribute to a deeper understanding of the interrelation of man and the universe, the individual and society, and of the values shared by all people." Dr. Dubos, one of the most distinguished scientists in America, has contributed the twenty-second volume.

In the first chapter, The Gardens of Eden, he develops the thesis that complete freedom from disease is incompatible with the process of living. He outlines the history of the "illusion" that perfect health and happiness are within man's possibilities. Whereas primitive religions and folklore were wont to place this paradise on earth in the remote past, more recently men have envisaged an era in the future in which man would be free from disease and old age, and death would be indefinitely postponed. The disappearance of leprosy and plague and the partial control of smallpox, malaria, and summer diarrhea gave justification for this optimism. After Charles Darwin, man no longer looked back with regret upon a Paradise Lost, but instead began to think that he was approaching the gates of the new Eden promised by the scientists. But even before laboratory medicine had effectively entered the picture, spectacular improvements in the sanitary and nutritional state of the Western world had been made by the humanitarians and social reformers of the 19th century. In contrast, modern medical science has made little practical progress toward controlling the diseases that were not dealt with by the nineteenth-century reformers. This lack of success in dealing with non-contagious disease is emphasized by the fact that, while the modern American boasts of the scientific management of his body and soul, his life expectancy past the age of 45 is hardly greater today than it was several decades ago. Dr. Dubos states on page 21 that 10 per cent of America's income goes for medical care, but, with some inconsistency, states on page 180 that 5 per cent of the national income was spent on medical and institutional care of disease in 1949, the only year in which he provides data. One tends to agree, however, that science has not been completely successful in "substituting precise knowledge for the lost instincts of health," that the creation of health and happiness transcends specialized knowledge of remedies and treatments and is determined by the manner in which the individual responds and adapts to the challenges met in everyday life.

Chapter II discusses "Biological and Social Adaptation." Using many examples, the author traces brilliantly specific physiological adaptations and adaptive patterns of behavior. He concludes that living creatures are highly integrated systems involving all forces of the environment, lifeless and living, atmospheric and geological. The dynamic equilibrium between living things and their environments changes endlessly. No permanent equilibrium is ever reached. Problems of physiological, social and ecological adaptations endlessly arise.

Chapter III considers "Struggle and Partnership in the Living World." Using his extensive microbiological experience as a backdrop, the author develops the concept of ecological equilibrium, the disturbance of which results in disease.

In the superb fourth chapter, he supports the concept that disease results from a disturbance in any of the factors of man's external or internal environment—be it weather conditions, availability of food, working habits, economic status, or emotional stress. In so doing, he rejects the doctrine of specific etiology of disease that had gained almost universal acceptance in medicine as a consequence of the work of Pasteur, Koch, and their followers. He does not question that modern medicine has been built upon the knowledge that microbial

agents, disturbances in essential metabolic processes, deficiencies in growth factors or in hormones, and physiological stresses are specific causes of disease. Rather he believes that these factors provide only an incomplete account of the causation of disease. The continual search for *the* cause of cancer, of arteriosclerosis, of mental disorders, and other great medical problems of our time, may be a hopeless pursuit, because most disease states are the indirect outcome of a constellation of circumstances rather than the direct result of single determinant factors. Because the process of living involves complex interrelationships, any pathological process is the resultant of a multiplicity of diverse influences, in both the internal and external environment. Furthermore, all phases of disease are affected by adaptive responses of the organism.

By equating disease with a single cause, the doctrine of specific etiology initially negated the Hippocratic view of health as equilibrium. The vague and abstract concepts of harmony have now re-entered the scientific arena, largely through the formulations of Darwin, Claude Bernard, Walter Cannon and their followers. An excellent case in point is the production of mammary carcinoma in certain inbred strains of mice. At least four factors are involved: the milk virus, a hormonal influence, a genetic susceptibility and a nutritional factor. The doctrine of multiple factors is both disheartening in its complexity and yet a source of hope, since the multiplicity of links in the chain of causation increases the chance of finding a weak part in the chain and thus discovering a method of control.

Chapter V discusses the never-ending oscillation between two points of view in medicine: the prevention of disease, symbolized by Hygeia and the treatment of disease, symbolized by Asclepius. A striking conclusion of microbiologist Dubos is that "The effect of antibacterial drugs is but a ripple on the wave which has been wearing down the mortality caused by infection in our communities."

In the remaining chapters, the author discusses in brief detail the problems of undernutrition and overnutrition, the effect of social patterns on diseases, such as tuberculosis, industrial smogs, "the pestilence that walketh in darkness," psychiatric illnesses, and, in the final chapter, "Utopias and Human Goals." His conclusion is that the intellectual concept of an idealized society, being a static view of the world, is incompatible with reality. "Man has never sought tranquility alone," wrote Sir Winston Churchhill. "His nature drives him forward to fortunes, which, for better or for worse, are different from those which it is in his power to pause and enjoy."

There is no one to whom I would not recommend this excellent book. It is only a pity that so many provocative ideas have to be compressed into such a brief volume.

HENRY N. WAGNER, JR.

Diseases of Laboratory Primates. By THEODORE C. RUCH. 600 pp., $7.50. W. B. Saunders Co., Philadelphia, Pa.

This book is the first of a projected four-volume *Handbook of the Primates*. Subsequent volumes will include a general description of laboratory primates, available normative data, and a bibliography of primatological literature, which will bring up-to-date the author's *Bibliographia Primatologica* of 1941.

Diseases of Laboratory Primates is planned primarily as a guide to diseases occurring in primate animal colonies. Diseases are classified, for the most part, by organ systems. Pathology, clinical symptoms, methods of diagnosis, treatment and prevention, are all discussed with full reference to the literature. The care with which detailed descriptions are presented may be illustrated by a case of central nervous system tuberculosis which I recently saw in a young rhesus macaque. Histologically, the lesions were somewhat unusual because the large central areas of necrosis were filled with leucocytes, without any frank caseation.

Ruch specifically notes this point as frequently characteristic of tubercles in the nervous system of primates.

Because the book is focussed primarily on diseases developing spontaneously in the laboratory, the material covered has been purposely restricted in two ways. First, no attempt is made to survey the literature on deliberately induced disease. For example, under poliomyelitis are discussed six instances of accidental or spontaneous infection in monkeys and chimpanzees, without reference to the vast literature on experimental poliomyelitis. Second, there is no systematic review of diseases occurring in the wild, except when they are brought into the laboratory by captive animals.

This is a pioneer attempt to write a definitive text of primate medicine. The great effort made to search out available data is manifest, and these data have been integrated in a lucid presentation. This volume will probably become a standard reference for the diagnosis and treatment of disease in non-human primates.

NEAL NATHANSON

Textbook of Surgery, 3rd edition: Edited by H. FRED MOSELY 1336 pp., $17.00. The C. V. Mosby Co., St. Louis, Mo.

This widely-used Canadian textbook of surgery appears now in a third edition enlarged both in scope and volume. It does not suffer from its provincialism, but rather maintains a style, clarity of expression and integration of purpose which reflects the close association of its forty McGill University faculty contributors. An excellent new section on Pediatric Surgery has been added, both compact and inclusive. The Chapters on Thoracic and Cardiac Surgery have been greatly expanded with excellent organization and concise presentation. The operative descriptions throughout the book are beautifully supplemented with the color plate drawings of Dr. Frank Netter of the Ciba Foundation. The bacteriology section is, if anything, too detailed for a surgical text. The discussion of endocrine responses to surgical trauma is cursory but the references are complete. The chapter on Isotopes and Irradiation is timely and very appropriate. The exhaustive bibliography accompanying each chapter remains a strong asset and in addition a listing of pertinent teaching films now rounds out the available reference material. The section on Amputations and Fractures is much more comprehensive than those in most surgical textbooks in this country. The book as a whole lacks the dynamic physiologic approach of several recent surgical texts but depends upon its emphasis on physical diagnosis and well integrated references to maintain its appeal as a fine surgical textbook and comprehensive reference work.

J. ALEX HALLER, JR.

The Plasma Proteins. Clinical Significance. By PAUL G. WEIL. 133 pp., $3.50. J. B. Lippincott Co., Philadelphia, Pa.

This monograph (122 small pages) and its equally contracted bibliography (49 references) proposes to assemble "as many of the facts as possible" pertaining to the clinical importance of plasma proteins. It is an assemblage of facts, many of which merit amplification and/or qualification which the brevity of the monograph does not permit. Its usefulness is limited by this brevity which tends to close issues far from solved or even understood. However, there is merit in again focusing attention on protein abnormalities, quantitative and qualitative, in a wide spectrum of disease states.

MARY BETTY STEVENS

Radioactive Isotopes in Clinical Practice. By EDITH H. QUIMBY, SERGEI FEITELBERG AND SOLOMON SILVER. 451 pp., $10.00. Lea & Febiger, Philadelphia, Pa.

A recent nationwide survey documented the tremendous growth of the use of radioisotopes in both clinical and research medicine. Last year 400,000 internal administrations of radio-

isotopes were given. At present 40 per cent of non-Federal hospitals have a radioisotope service, and it is estimated that 60 per cent will have one by 1970. Twenty-seven per cent of medical school research projects use isotopes in human beings. Such data as these, recently provided by the Atomic Energy Commission, clearly indicate that medical education must include training in nuclear physics, radiochemistry, and both clinical and public health aspects of nuclear medicine.

The present volume is one of the best available textbooks on the clinical use of radioisotopes. Since 1954 the authors have given a four-week full-time course in the uses of radioisotopes in clinical practice. The book is the outgrowth of their lectures, laboratory exercises and conferences during the course. Each of three separate units of approximately equal length—Basic Physics, Instrumentation and Laboratory Methods, and Clinical Applications—has been written by the person responsible for that particular part of the program. The inclusion of basic physics and instrumentation in moderate detail is a feature of the book that is an improvement upon other available radioisotope texts. The chapters on instrumentation are written to enable the reader to have an understanding of the function of commonly used equipment without excessively detailed consideration of their design and circuitry. Radiation hazards and disposal of radioactive waste are very adequately presented.

In the section on clinical applications, preponderant stress (80 of 149 pages) is given to the diagnosis and treatment of thyroid disorders, especially hyperthyroidism. This was done since most physicians in the training course were primarily interested in the use of iodine^{131}. The chapters on the thyroid could have been written only by a physician with wide experience in thyroid disease. For example, the discussion of the pitfalls in the interpretation of diagnostic studies with I^{131} is excellent. Despite the emphasis of the book on radioisotopes, the author laments the unjustified disrepute into which the determination of the basal metabolic rate has fallen. Another example of the author's clinical perspective is the statement, "The clinician who allows himself to be dominated by a laboratory technique, new or old, is doomed to disaster." This approach gives a true balance to a textbook on radioisotopes.

Unfortunately, the other chapters on clinical applications suffer by comparison with the thyroid work. These are too sketchy to be of real value, other than providing the briefest survey of the subjects.

In summary, this book fulfills the need for a textbook on the use of radioisotopes in clinical medicine. The advances in this field are so rapid, however, that new textbooks will be required frequently.

HENRY N. WAGNER, JR.

The Central Nervous System and Behavior. Transactions of the First Conference, Feb. 1958. Edited by MARY A. B. BRAZIER. Sponsored by the Josiah Macy, Jr. Foundation and The National Science Foundation. 450 pp., $5.25. The Josiah Macy, Jr. Foundation Publications, New York, N. Y.

This is a report of a conference of twenty-nine investigators representing the various disciplines interested in the correlations between cortical phenomena and behavior. The purpose of the conference was not only to exchange information, but to improve understanding, broaden perspectives, change attitudes, and overcome prejudices. The result is a four hundred and fifty page text with some new information, an excellent summary of the work in certain areas and an illustrated history of Russian physiology and medicine.

The initial presentation on Russian contributions is an excellent survey of some important historical figures in the area from Sechenov to Pavlov. Following is a review of post-Pavlovian work so brief as to be misinformative. There is scanty mention of contributions of Gantt and his co-workers at Johns Hopkins and of contemporary Russian and Euro-

pean workers. Konorski, not Skinner, is given credit for the introduction of instrumental conditioning procedures. There is little to indicate the great variety of responses which have been conditioned or of the more recent work on the effect of person (original with Gantt) on the acquisition of conditional responses.

Leese poses three issues (1) What are the brain correlates of conditioning? (2) Behaviorally, what constitutes conditioning? (3) What are the relationships between conditioning and learning? These could have served as the major topics of the conference. Unfortunately, these are neglected by other participants. There is an excellent section on brain stimulation and conditional reflexes, contributed mainly by Doty but which is unnecessarilly complicated with too general or particular issues. Morell presents some excellent information on the EEG and conditioned learning. There is a review of the work of Galambos and others on the electrical correlates of conditioning and a final summation by Livingston of NIMH.

The problems of research in the area of nervous system and behavior may be best expressed in a radio communications analogy: one investigator discovers that there are precise bands of electronic emission (stations), another disregards this and studies the modulations imposed upon it (the program). A third has found that cutting certain wires (circuit) produces marked changes in output; squeals, hum and silence. The three scientists then proceed to discuss, interrelate their findings, and infer the nature of the transmitter and receiver from their data. What is lacking, obviously, is the key bit of information concerning the basic properties of electronic transmission and reception. Without this guiding principle, basic facts may not be inferred. More important, it may never be possible to discover true facts and relations of the system by this approach from the bottom upwards. It is only after a comprehensive grasp of system theory that the bits and pieces of facts concerning system *operation* and circuitry become comprehensible.

The impression with which the reader leaves the report of this conference is that new instrumental techniques for studying cortical functioning during conditioning and learning are being employed and that potentially, these may lead to greater insights on brain function. Equally impressive however, is the fact that the new generation of behavioral scientists is displaying a healthy consideration for the tangible, objective fact.

Although it is debatable whether contemporary electrophysiological approaches will lead to a complete understanding of cortical function, the renewed interest in fact, not fancy, is an encouraging sign.

CLINTON C. BROWN

Emotional Problems of Childhood. Edited by SAMUEL LIEBMAN. 176 pp., $5.00. J. B. Lippincott Co., Philadelphia, Pa.

This book, consisting of lectures given at the North Shore Hospital is aimed at the general practitioner. It covers such subjects as psychological aspects of pregnancy, early parent-child relationship, feeding, sleep and motility in infants, learning difficulties, misbehavior and social maladjustment of the school child, management of puberty, sex and neurosis in adolescents, and the emotional problems of the physically handicapped. Particularly noteworthy are the chapters by Therese Benedek, Joseph Cramer, Julius Richmond, and Earle Lipton, and the one by Eugene Falstein.

With such variety of topics the nine lecturers were able to cover each subject only briefly, but succeeded in giving theoretical considerations as well as practical suggestions for the handling of those problems.

The editor, Dr. Samuel Liebman, believes—and this reviewer wholeheartedly agrees—that the medical practitioner should and must treat minor emotional problems. More seminars like the one in Chicago to which these authors have contributed would enable the

pediatrician to be thoroughly informed about the problems that he faces in his everyday practice.

CAROLA GUTTMACHER

Applied Medical Library Practice. By THOMAS E. KEYS. With chapters by CATHERINE KENNEDY AND RUTH M. TEWS. 495 pp., $10.75. Charles C Thomas, Springfield, Ill.

The author is Librarian of the Mayo Clinic, Rochester, Minnesota, and Assistant Professor of the History of Medicine of the Mayo Foundation, University of Minnesota.

Mr. Keys' book is directed toward the physician, the medical student, and others who use the medical library. In the preface Mr. Keys indicates that the book is designed to teach the physician how to use the library. Besides accomplishing his purpose in this respect, Mr. Keys has produced a book which also serves as an introduction to medical history and as a reference book for information and facts about medical literature and medical libraries.

In the first few chapters he introduces the reader to the administrative and technical aspects of medical librarianship, discussing staff, library departmentalization, acquisition of library materials, cataloguing, and classification. Next, he describes the important medical indexes and abstract journals, presenting helpful suggestions for their use. Of particular interest to persons writing in the medical field is the chapter on the fundamentals of medical bibliography.

In the chapter entitled "Reprints and Their Orderly Arrangement" Mr. Keys states that in his experience collected reprints of medical schools and departments are generally little used in a medical library. This view will be sustained by a large majority of medical librarians. For small libraries and for individual physicians, however, Mr. Keys points out that reprint collections may have real value and suggests ways of organizing them.

Miss Ruth M. Tews, a co-author, presents a very valuable chapter on the patients' library, covering its early history, organization, and concluding with some remarks on bibliotherapy.

Special attention should be drawn to two short chapters in which the author, discussing the place of the medical library in graduate medical education and research libraries in medicine, has set forth what might be called the philosophy of medical librarianship.

The second half of the book is largely historical. Beginning with a chapter describing some of the important private libraries that physicians have collected, continuing with descriptions of some of the larger medical libraries in the United States, and a brief history of medical publishers and medical books through the 19th century, Mr. Keys concludes with a look into the future in his chapter, "Changing Concepts in Library Services".

The five appendices are valuable to both physicians and medical librarians. They are:

1. Names and addresses of publishers of medical and related books in the United States: arranged by city.
2. Names and addresses of Medical Bookdealers in the United States who handle new books: arranged by city.
3. Names and addresses of antiquarian Booksellers in the United States dealing in medical and related subjects: arranged by city.
4. Titles of journals listed in rank order of the top journal circulation of unbound issues for 1954–1956 in the Mayo Clinic Library.
5. A bibliography of medical works in facsimiles.

JOHN M. WHITTOCK, JR.

BOOKS RECEIVED FOR REVIEW

Anatomy and Physiology. Vol. I: Cells, Tissues, Integument, Skeletal, Muscular and Digestive Systems, Blood, Lymph, Circulatory System. (College Outline Series.) By EDWIN B. STEEN AND ASHLEY MONTAGU. 332 pp., $2.50. Barnes & Noble, New York, N. Y.

Bigger's Handbook of Bacteriology for Students and Practitioners of Medicine, 7th edition. By F. S. STEWART. 611 pp., $8.00. The Williams & Wilkins Co., Baltimore, Md.

Clinical Dermatology. For Students and Practitioners. By HARRY M. ROBINSON, JR. AND RAYMOND C. V. ROBINSON. 242 pp., $8.50. The Williams & Wilkins Co., Baltimore, Md.

Clinical Orthopaedics No. 13. The Hand, Part I. Edited by ANTHONY F. DEPALMA. 393 pp., $7.50 single copies. J. B. Lippincott Co., Philadelphia, Pa.

Diseases of the Nose, Throat and Ear, 2nd edition. Edited by CHEVALIER JACKSON AND CHEVALIER L. JACKSON. 886 pp., $20.00. W. B. Saunders Co., Philadelphia, Pa.

History and Trends of Professional Nursing, 4th edition. By DEBORAH MACLURG JENSEN. 610 pp., $5.25. The C. V. Mosby Co., St. Louis, Mo.

La Biologie des Homogreffes, Vol. LXXVIII. 284 pp. Colloques Internationaux du Centre National de la Recherche Scientifique, Paris, France.

Logan Clendening Lectures on the History and Philosophy of Medicine, 7th Series. The Meaning of Poison. By LLOYD G. STEVENSON. 53 pp., $2.00. University of Kansas Press, Lawrence, Kans.

Medicinal Chemistry, Vol. IV. By WILBUR J. DORAN. Edited by F. F. Blicke and R. H. COX. 334 pp., $12.00. John Wiley & Sons, New York, N. Y.

Microbiology. Yesterday and Today. Edited by VERNON BRYSON. 122 pp., $4.00. Institute of Microbiology, Rutgers University, New Brunswick, N. J.

Preventive Medicine. Principles of Prevention in the Occurrence and Progression of Disease. Edited by HERMAN E. HILLEBOE AND GRANVILLE W. LARIMORE. 731 pp., $12.00. W. B. Saunders Co., Philadelphia, Pa.

Speech and Brain-Mechanisms. By WILDER PENFIELD AND LAMAR ROBERTS. 286 pp., $6.00. Princeton University Press, Princeton, N. J.

Surgery of the Foot. By HENRI L. DUVRIES. Foreword by KARL A. MEYER. 494 pp., $12.50. The C. V. Mosby Co., St. Louis, Mo.

Synopsis of Treatment of Anorectal Diseases. By STUART T. ROSS. 240 pp., $8.50. The C. V. Mosby Co., St. Louis, Mo.

Textbook of Pediatrics, 7th edition. Edited by WALDO E. NELSON with the collaboration of 81 contributors. 1462 pp., $16.50. W. B. Saunders Co., Philadelphia, Pa.

That the Patient May Know. An Atlas for Use by the Physician in Explaining to the Patient. By HARRY F. DOWLING AND TOM JONES assisted by VIRGINIA SAMTER. 139 pp., $7.50. W. B. Saunders Co., Philadelphia, Pa.

Trauma. By HARRISON L. MCLAUGHLIN. 784 pp., $18.00. W. B. Saunders Co., Philadelphia, Pa.

CENTRAL NERVOUS MECHANISMS SUBSERVING POSITION SENSE AND KINESTHESIS[1]

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It is a general observation that afferent information in the somatic sensory sphere plays an important though not an exclusive role in the perception of the position of the body in space. This sense of position includes also perception of the passive attitudes of the extremities, and of changes in joint angles during movement of the limbs, which latter we term kinesthesis. The defect in position sense and kinesthesis so characteristically a part of the syndrome seen in humans with lesions of the dorsal columns of the spinal cord indicates that afferent impulses transmitted via neural elements of the dorsal column-medial lemniscal system are prepotently involved in evoking these forms of sensation. The physiological problems, not new, are to determine the receptor organs concerned as well as their adequate stimuli, and to describe so far as is possible the central nervous events set in motion by such stimuli.

The thesis presented in this paper is that the receptor organs of the joint capsules and pericapsular tissue play a major role in position sense, together with their afferent projections into the dorsal columns, their related lemniscal and thalamic relay neurons, and that class of nerve cells of the postcentral gyrus upon which neural activity set up by joint rotation finally impinges. At each successive relay and in the postcentral gyrus itself neurons activated by these joint receptors are arranged in topographic patterns mutually interlocked with those representing the cutaneous sensory sheet. Such a thesis was indicated by the results of earlier studies upon the somatic sensory cortex of the cat (33), and it has been confirmed and extended in this present study of the postcentral gyrus of the macaque monkey, carried out with the method of single unit analysis.

Data are presented below which describe the number and locations of cortical neurons activated by joint rotation, which we have studied, together with a description of the relation between the response patterns of these cells and the peripheral stimuli which drive them. Pairs of adjacent cells have been observed which are reciprocally related to a particular joint, a form of afferent reciprocal innervation. A description is given of a form of cross-modal interaction, in

[1] Aided by grant No. B-1045 from the National Institutes of Health, U. S. Public Health Service.

[2] Fellow of the Rockefeller Foundation, from the Department of Human Anatomy, Oxford, England.

which cortical neurons activated by joint rotation are inhibited by stimulation of the skin. The physiological significance of this interaction is obscure.

METHODS

The data presented here are taken from experiments upon the 27 monkeys which form the basis of the preceding paper. Details of the experimental methods are given in that report: the method of recording from single cortical neurons, histological study of serial sections of the experimental brains, and reconstruction of electrode tracks, etc. Indeed, information obtained by study of postcentral neurons sensitive to joint rotation has already been used in that study in formulating a description of the functional organization of the postcentral gyrus.

Joints were rotated by hand, and we have available only relatively crude indices of the onset and velocity of movements. The association of the responses observed to the movements occurring was so obvious, however, that we believe conclusions based upon the assumption of that relation are valid.

The impulse frequencies of the discharge of each cortical neuron studied were counted in each successive short interval of time (100, 200, or 400 msec.) and plotted against time as running averages of frequencies per second. As compared with plotting the reciprocals of impulse intervals against time this method gives a better impression of the overall level of activity, but because of it the frequencies actually reached during the initial phase of movement are slightly higher than they appear in our graphs.

RESULTS

I. General Considerations

(1) *The validity of the submodality identifications*

The identification of joint movement as the adequate driving stimulus for certain cells of the postcentral gyrus is the essential datum upon which that to follow is based. The further conclusion is drawn that the receptors involved are those of the joint capsules and the pericapsular connective tissue, for we have not observed cells of the postcentral gyrus to be driven by stretch of muscle. That the first was indeed the case was demonstrated directly by dissection of the joint while a cortical neuron previously identified as a "joint unit" was under study. Such experiments have shown, without exception, that the mechanical stimulation of the tendon grooves, the joint capsule, and the pericapsular connective tissue produced increases in the discharge rate of the cell under study. For example, near the end of experiment M7 a neuron was isolated (7-3) which was sensitively responsive to flexion of the contralateral elbow. It was unaffected by stimulation of the skin of the contralateral arm—or indeed of anywhere else—or by mechanical stimulation of the deep tissues of the arm other than those of the joint. It was decided to examine the elbow region by direct dissection, at the conclusion of which the following note was written.

The tendon of the triceps was exposed through a posterior midline incision extending down over the olecranon of the ulna. The tendon was incised from its lateral aspect, and the periosteum of the humerus and the capsule of the joint exposed. Driving of the neuron was evoked by pressure on the posterior part of the capsule only, and not by touching the periosteum of the condyles of the humerus or the articular surfaces inside the joint, nor by tugging on the sectioned triceps tendon. A further exposure was made of the capsule on the anterior aspect of the joint, to determine whether pressure there would inhibit the spontaneous activity or evoked responses of the cortical neuron. Such an inhibition was not found, but this was not an unequivocal observation, for nerve branches to the anterior capsule may have been damaged by the dissection.

Such observations as this have been made in only a few cases. In general the identification of the "joint units" was based upon: (a) the sensitive and direct relation of the change in frequency of discharge of the cortical cell under study to the movement and the steady angle of the related joint, (b) the fact that such neurons are not excited by direct stimulation of the skin, and (c) the fact that they are equally insensitive to the direct manipulation of muscle tissue. All our data fit with the conclusion stated above, and none are contrary. We will proceed, therefore, on the presumption that such submodality identifications are valid. Finally, we have not observed the submodality type to change.

In all, we have isolated and studied the responses of 325 neurons of this class, which form a part of the total population of postcentral neurons described in the preceding paper. Photographic records of the responses of 118 of these cells have been made. Most of those studied were located in the arm and shoulder region of the postcentral gyrus, as indicated by the joints to which they were related: shoulder, 103; elbow, 86; wrist, 75; fingers, 20; vertebral column, 2; hip, 25; knee, 5; ankle, 3; and toes, 6. We have not observed any neurons that were activated by rotation of joints of the ipsilateral side of the body.

(2) *Locations in the cortex of neurons responsive to joint rotation*

In the preceding paper a gradient in the submodality types of neurons encountered in areas 2, 1 and 3 of the postcentral gyrus was described (see Figs. 2 and 3 of that paper). It is a question of some interest whether this gradient depends upon a differential distribution of those neurons related to the joints as well as of those related to other deep tissues, both of which were considered together in the study referred to. The chart of Figure 1 indicates that when the distribution of joint neurons, only, is compared with that of the skin group, an even more striking differential is revealed. On the other hand, the distribution of joint neurons of areas 2 and 1 in depth in the cortex is quite similar to that of the total population of neurons studied, or of the entire deep group, which is shown in Figure 1 of the preceding paper.

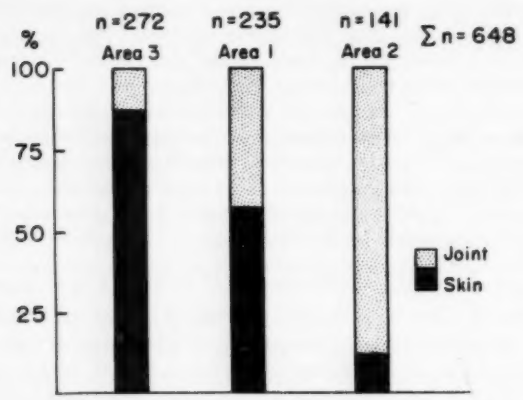

FIG. 1. Bar graph indicates the relative prevalence in each cytoarchitectural area of the postcentral gyrus of neurons sensitive to cutaneous stimuli and those activated by the movement of joints. Only neurons encountered in electrode penetrations whose tracks were later identified in the serial sections have entered these populations.

(3) *Relation of the receptive fields of deep joint neurons to the central projection pattern of the body form*

In this regard the joint whose movement activates a given neuron is considered to be the peripheral receptive field of the cell. We have made a detailed analysis of the receptive fields of joint neurons observed in penetrations made at various mediolateral levels of the postcentral gyrus. When this pattern is compared with that for neurons sensitive only to stimulation of the skin, a remarkable fact emerges. That is, that the two patterns are, with minor differences, very similar. They are interdigitated in a single image of the body form. Evidence presented previously indicates that neurons related to each submodality are arranged in vertical columns of the cortex, and that adjacent columns are fitted together to form the mosaic of the cortical projection pattern. Within a given mediolateral region a portion of the body is represented in an antero-posterior strip. The posterior portion of this strip—that in area 2—is devoted almost solely to the representation of deep tissues, with only a rare column for skin receptors interposed. As one moves anteriorly across area 1 and into area 3 the proportion gradually shifts to one in which the reverse holds true, in which neurons and columns related to skin receptors far outweigh those related to deep receptors. Such at any rate is the most likely conclusion indicated by the observations made in the present studies.

II. Patterns of Activity of Cortical Neurons Evoked by Joint Movement

Once a cortical neuron has been isolated and identified as sensitive to movement of a joint, one observes a rather precise relation of its activity to the

angle of the joint. For example, Figure 2 shows the discharges of a neuron sensitive to flexion of the contralateral great toe upon the first metatarsal. The receptors concerned almost certainly lay in the joint capsule. With the toe in the neutral, or anatomical, position the neuron was silent, or discharged impulses at a very low and irregular rate. Record A of Figure 2 shows the response evoked when the toe was twice moved briskly into and out of sharp flexion. Record B of Figure 2 shows the sustained discharge of this neuron at a somewhat lower rate when the toe was moved into the flexed position and held there for a period of about four seconds.

We do not possess precise quantitative data concerning the thresholds (i.e., the speed and angular displacement of joints required to excite) of neurons such as that of Figure 2. All of our observations fit the notion, however, that such cortical cells begin to discharge at an absolute value of angular displacement. On the other hand, the speed with which the joint is moved into the excitatory angle determines the *frequency* of the onset transient discharge of the neuron. The final steady adapted rate of discharge depends upon absolute joint angle. With such a neuron it is not possible—no matter how slowly the movement is carried out—to bring the joint into the excitatory angle and to the maximal excitatory position without producing an increase in the discharge rate of the cell.

(1) *Adaptive patterns*

Such a constancy of behavior is not observed when the joint is held steadily within the excitatory angle. The record of Figure 3 illustrates one type of behavior. This neuron was driven by extension of the contralateral ankle. Quick movement of the joint from the neutral position into full extension produced a precipitous acceleration of the firing rate of the cell to a high level, an

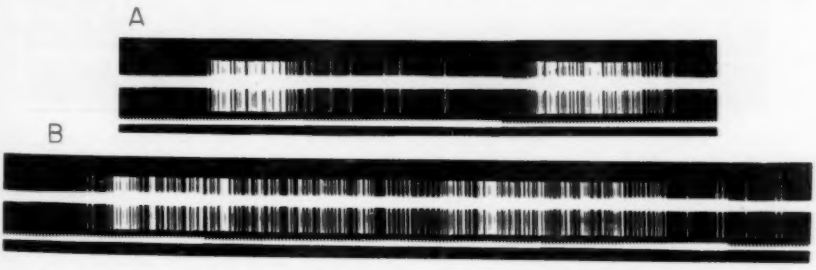

FIG. 2. Impulse discharges of neuron 24-12, evoked by brisk lateral deviation of the contralateral toe at the metatarso-phalangeal joint. Responses in A evoked by two brisk lateral deviations, those in B by a sustained lateral deviation. Neuron isolated 880 μ below the cortical surface of area 1, during penetration M12-P6, and studied for 20 minutes. Time line 40 per second; amplitude of spikes about 4 mv. Short time constant recording.

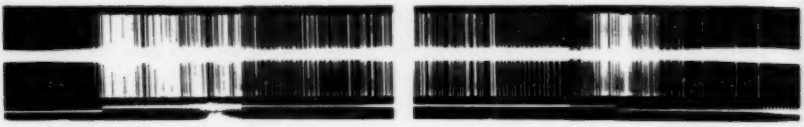

FIG. 3. Impulse discharges of neuron 24-13, evoked by the movement of extension of the contralateral ankle joint. Movement produced a very high frequency onset transient discharge, which fell rapidly to a more or less steady plateau level as the joint was held in full extension. Movement back to the neutral position produced a second transient acceleration in discharge, which, upon completion of the movement, fell to a low level. Break in record indicates several seconds of steady state discharge not shown. Total duration of the movement was 13 seconds; onset and cessation indicated by reversals of the time line, which is 40 per second; amplitude of spikes 4–5 mv. Cell isolated 2490 μ below the cortical surface in area 3 during penetration M24-P6, and observed for about 20 minutes. Short time constant recording.

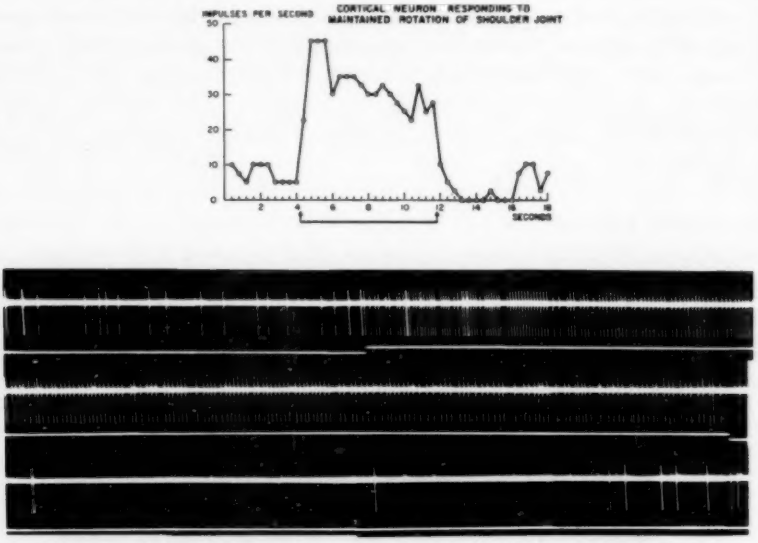

FIG. 4. The graph above plots the impulse frequency of neuron 18-3 before, during, and after an external rotation of the contralateral shoulder joint, which was maintained for 8 seconds. Records of impulses shown below. Onset and cessation of movements to and from the externally rotated position are indicated by reversals of the time line, which is 40 per second. Short time constant recording; spikes about 2 mv. amplitude. Cell isolated 1090 μ below the cortical surface in area 1 during penetration M18-P2. Observed for only 10 minutes.

onset transient. During maintained extension the frequency of discharge fell slightly to a new and almost level plateau, the *steady state discharge*, which was maintained at this level for the 13 seconds of steady extension. With return of the ankle to the neutral position the frequency transiently increased

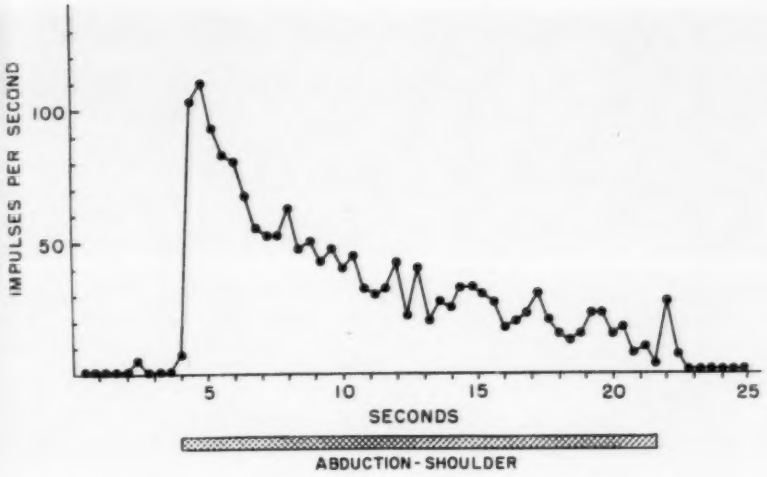

FIG. 5. A graph of the impulse frequency of neuron 9-7 before, during, and after a steadily maintained abduction of the contralateral shoulder which lasted for about 16 seconds. The frequency slowly fell from its high initial level to a low level, and had not yet reached a steady rate when the shoulder was adducted. Cell isolated 4300 μ below the cortical surface, in area 3, during penetration M9-P1; observed for 40 minutes.

again, then fell abruptly to a low level. Somewhat similar behavior is shown for other cells by the records and chart of Figure 4, and the charts of Figure 8.

Such a maintained discharge during steady displacement of a joint in the excitatory direction is a common event, but it is by no means the only pattern observed. The chart of Figure 5 illustrates another. In this case the neuron responded to a rapid abduction of the contralateral shoulder with a rapid onset transient, the frequency of discharge reaching a level of more than 100 impulses per second during the first second of joint displacement. Thereafter, in spite of steadily maintained abduction, the frequency declined in a more or less exponential manner to a low plateau. With return of the shoulder to the neutral position the frequency of discharge fell to zero.

These two types of adaptive behavior occur in the large majority of cortical neurons driven by joint rotation. Others may discharge only a short burst of impulses when the joint moves into the excitatory angle, and repeat with a second burst during movement out of that angle, i.e., such quickly adapting neurons may fire to movement in either direction. They have been observed rarely; one example is given in Figure 6, in this instance of a neuron driven by flexion of the contralateral elbow. It is not uncommon to observe the discharges of two neurons in the record, each responsive to the movement of the same single joint in the same direction, the one displaying a transient and the other a sustained discharge in response to that movement. Still other neurons show

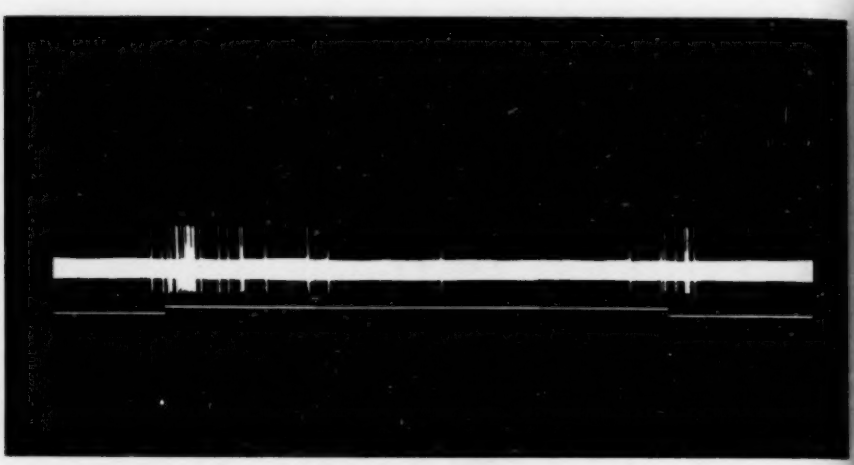

FIG. 6. Impulse discharges of neuron 13-8, activated during the transient phases of movement to and from full flexion of the contralateral elbow. Discharge adapts rapidly to extinction during the maintained steady position. Onset and cessation of movement indicated by the second line. Movement lasted 3.3 seconds, which gives time; amplitude of spikes about 0.5 mv.; short time constant recording. Cell isolated 670 μ below the cortical surface of area 2, during penetration M13-P3, and observed for 15 minutes.

intermediate rates of adaptation to steady joint displacement, and one of this type is illustrated in Figure 7. The elements of the population of cortical neurons driven by movement of a given joint occupy positions along a spectrum ranging from the steadily maintained plateau type discharge (in the majority) to the sharp on-off transients such as that illustrated in Figure 6. Some 81 per cent of those we have observed belong to the slowly adapting group.

(2) *Excitatory angles*

About 80 per cent of the neurons activated by joint displacement were sensitive over a considerable range of motion, "subtending" very wide excitatory angles. For joints with only one axis of movement such elements usually begin to discharge as the limb is displaced by even a few degrees from its neutral position. The steady adapted rate of discharge increases as the displacement of the joint is increased, and maximal rates of discharge are commonly produced by maximal displacement, e.g., by full flexion or full extension of the elbow or knee, without rotation. The remainder (14 per cent) were sensitive to joint position within or movement through narrow excitatory angles, at times as small as 20 degrees. When the joints to which such neurons are related are held steadily within these narrow excitatory angles, different neurons of this group display the variety of this adaptive property described above for wide

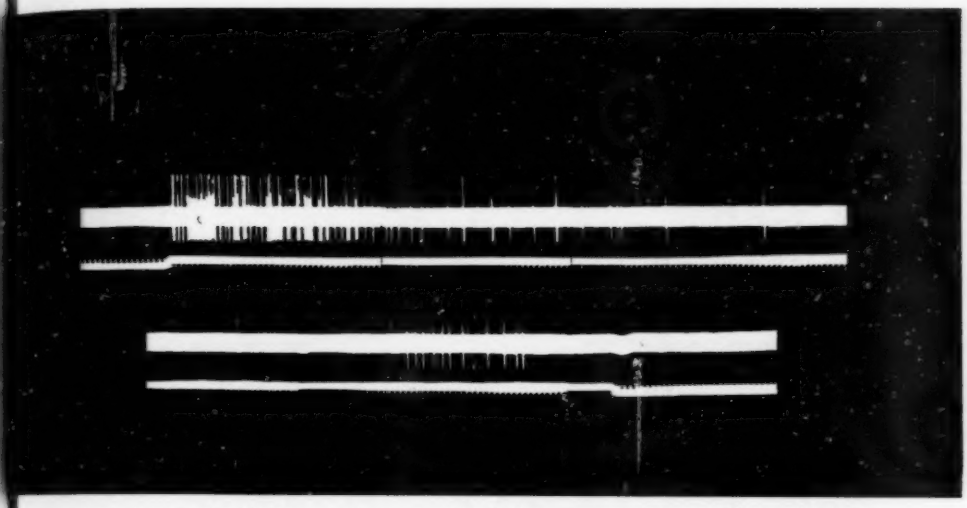

FIG. 7. Impulse discharges of neuron 13-9 during movement to, steadily maintained, and movement away from full flexion of the contralateral elbow. Onset and cessation of movement indicated by reversals of the time line, which is 40 per second. Amplitude of spikes is about 0.5 mv. Onset of movement produced a high frequency discharge which slowly adapted to extinction during steadily maintained flexion; the neuron discharged a short low frequency train as the movement away from flexion was begun. Cell isolated 950μ below the cortical surface of area 2 during penetration M13-P3, and observed for 20 minutes.

angle neurons. Cortical cells related to polyaxial joints such as the shoulder subtend solid cones rather than two dimensional angles. Position of the shoulder anywhere within this three dimensional cone produces an acceleration of discharge over the background rate. Usually, however, movement in only one direction within the solid angle will produce the maximal rate of discharge.

The question arises whether neurons which subtend very wide angles and those subtending narrow ones show any differential distribution in the three cytoarchitectural areas of the postcentral gyrus. Table I shows the distribution for 90 neurons which were studied carefully to determine this property, and whose locations in the cortex are known from the study of serial sections.

So far as such a number of observations may be considered reliable, then, there is some differential distribution of neurons in the cortex according to this functional property. It should be remembered, however, that in the lightly anesthetized state the cells of area 2 seem on occasion to be less securely linked to the peripheral driving stimulus than are those of area 3. It is just such a diminished synaptic security that one would expect to depress excitability of the system so that only the rapid onset transient of discharge would appear, and the steady state discharge be suppressed.

TABLE I

Cytoarchitectural Distribution of Postcentral Neurons Subtending Narrow Angles of Joint Rotation

Area	Total No. of Neurons	No. with Narrow Angles
2	31	13
1	41	5
3	18	2

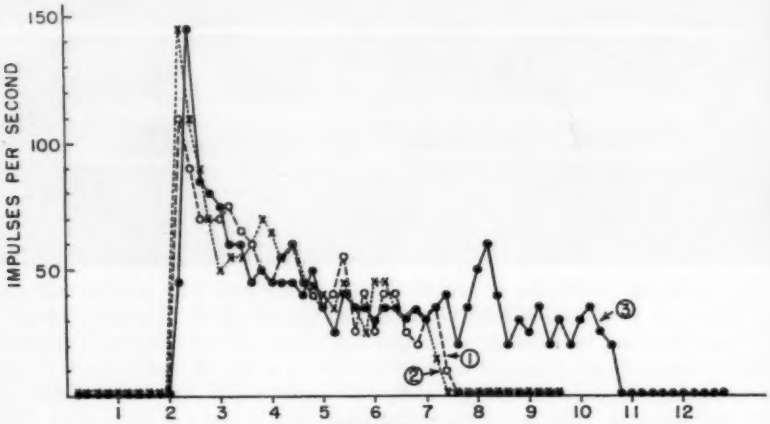

FIG. 8. Graphs plot the impulse frequency of neuron 9-8 before, during and after three abductions of the contralateral shoulder; neuron silent when shoulder in the adducted position. Movement into abduction produces onset transients of nearly 150 impulses per second; frequency declines from that peak to a more or less steady rate of about 30 per second, as shown by trial 3. Trials 1 and 2, of about equal duration, were ended before steady state reached. Similarity of pattern of activity evoked by the three trials is striking, even when joint rotation is produced by crudely controlled movements. Cell isolated 4440 μ below the surface of the cortex, in area 3, during penetration M9-P1, and observed for 40 minutes.

(3) *The fidelity of the response of cortical neurons to joint rotation*

A concept of the neural mechanisms depicting bodily movement and position such as that developed in this paper places upon the system one severe constraint. It requires that the receptors, afferent fibers, relay neurons, and cortical elements should display more or less the same discharge patterns when more or less identical movements occur, or when similar attitudes are assumed. The beautiful experimental results of Boyd and Roberts (5) and of Skoglund (38) have shown such a constancy for the joint receptors. We have tested this

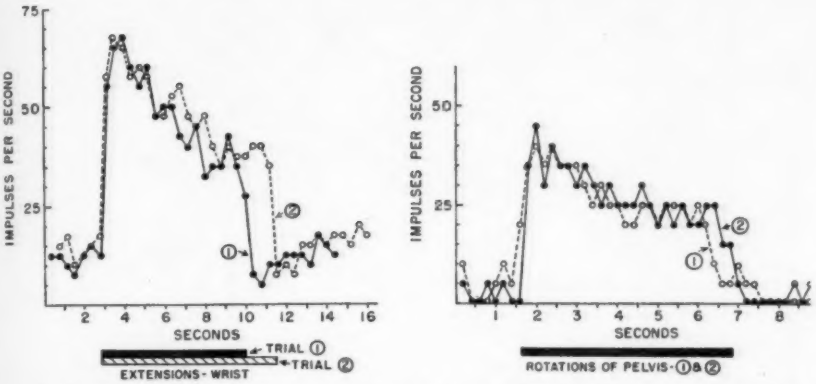

FIG 9. *Left.* Graph plots impulse frequency of neuron 14-5 during two extensions of the contralateral wrist. Replication of activity pattern is almost exact. Cell isolated 2650 μ below the cortical surface, in area 3, during penetration M14-P3; observed for 20 minutes. *Right.* Graph plots impulse frequency of neuron 15-8 during two rotations of the pelvis on the spine. Replication of the activity pattern is almost exact. Cell isolated 3200 μ below the cortical surface, in area 3, during penetration M15-P7; observed for 60 minutes.

property for a large number of cortical neurons. The results of one such experiment are shown by the charts of Figure 8. This neuron was activated by abduction of the shoulder; it was completely silent with the shoulder in the fully adducted position. Brisk movement into full abduction produced a rapid onset transient, the discharge frequency reaching 150 per second. From this peak the rate fell more or less exponentially to a steady level of 20–30 impulses per second. The figure shows that for the first two trials, of equal durations, the frequency curves almost exactly superimpose. For the third trial the abduction was maintained longer, to show the true plateau level, yet the first part of its course is a replica of that for trials 1 and 2. Charts for two other units studied in a similar manner are shown in Figure 9.

The charts of Figure 10 illustrate the results of a somewhat different experiment. This neuron showed an irregularly maintained discharge when the shoulder was held fully adducted. On three occasions, after the more-or-less level discharge rate had been reached with the arm held in this position, the shoulder was sharply abducted, held in that position for four seconds, and then quickly returned to the adducted position. The changes in frequency of discharge are shown by the three superimposed graphs of Figure 10. During each trial the neuron remained silent with the shoulder abducted. Subsequent adduction produced a fresh onset transient, followed by the early phase of decline to the adapted level. The question arises of whether the complete silence produced by joint movement in a direction opposite to that which excites is due only to unloading of the related peripheral receptors, or whether

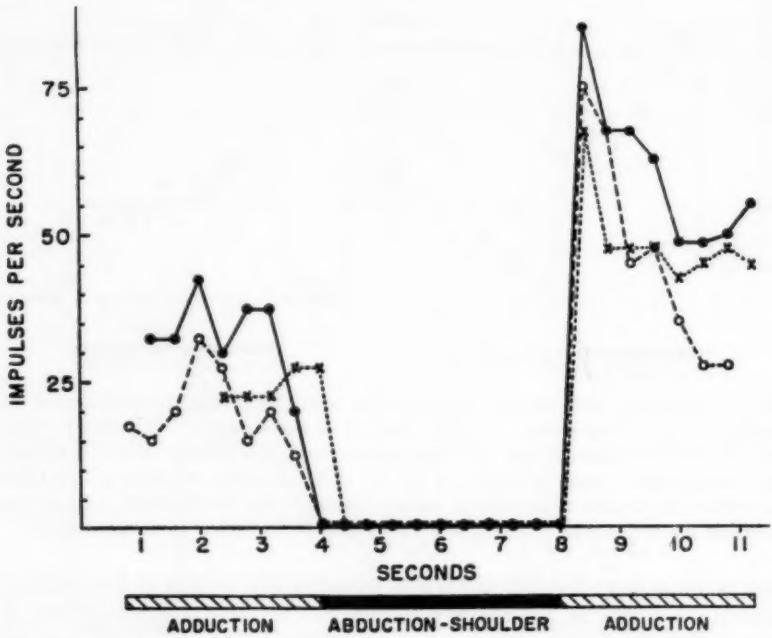

FIG. 10. Graphs plot impulse discharge rate of neuron 10-8. For each of the three trials the contralateral shoulder was first held for some time in the fully adducted position, which was the maximally excitatory position for the cell. The shoulder was then quickly abducted, held in that position for about 4 seconds, and then once again adducted. The complete cessation of activity when the shoulder was fully abducted is thought to result from reciprocal afferent inhibition, as well as to receptor unloading. Fresh movement to adduction produced in each case a new onset transient; only the early phases of the declines from those peaks shown. Patterns replicate from trial to trial. Cell isolated 5925 μ below the cortical surface, in area 3, during penetration M10-P4, and observed for 20 minutes.

afferent inhibition accounts, at least in part, for this event. This matter will be considered further below.

(4) *Afferent reciprocal innervation*

On 21 occasions we have isolated simultaneously in the record the action potentials of two cortical neurons, which responded in a reciprocal manner to alternating movements of the joints to which the two were related. The occurrence of such reciprocal action is, however, a very common event, and it has been observed many times when the discharges of the two units could not be isolated sufficiently for photographic records to be made. The graphs of Figure 11 indicate that neuron 10-15 was activated by extension of the contralateral wrist, and that the cell continued to discharge during this sustained

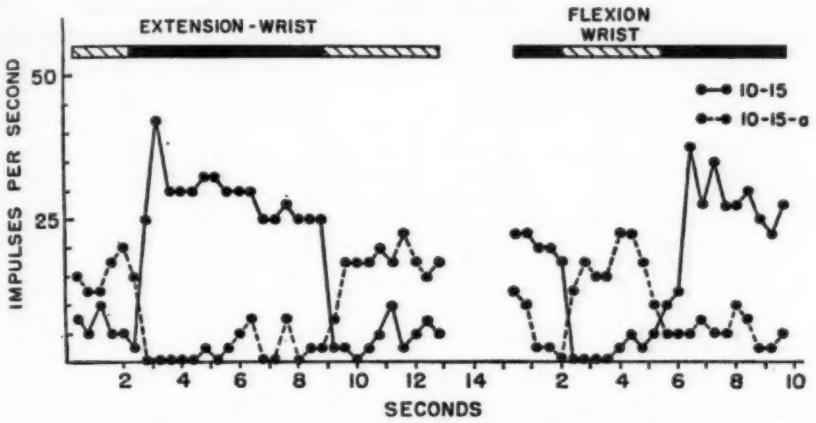

FIG. 11. Graphs plot the impulse frequencies of neurons 10-15 and 10-15-a, to the left during extension of the contralateral wrist and, to the right, during flexion. The first neuron is driven by extension, and its rate falls to zero upon flexion. Neuron 10-15-a shows a reciprocal pattern. Cells isolated simultaneously 2830 μ below the cortical surface, in area 3, during penetration M10-P5, and observed for 20 minutes.

joint displacement. Neuron 10-15-a, on the other hand, discharged at a steady rate with the wrist in the flexed position, and its discharge rate dropped to zero when the wrist was extended. The reciprocal behavior of these two neurons is obvious. In each case the neuron inactivated by the movement did not remain completely silent during the sustained displacement away from its excitatory direction, but after a few seconds began to discharge at a low rate. Such an event is similar to the escape from a steady inhibitory stimulus, such as that shown in Figure 14. It fits with the notion that the complete suppression of the discharge of a cortical neuron which occurs when the joint to which it is related moves away from its excitatory direction (see also Figure 10) is due to an active inhibitory process, as well as to simple receptor unloading. This reciprocal relation is shown also by the graphs of Figure 12, which plot the impulse frequencies of another pair of cells which were reciprocally related to flexion and extension of the contralateral hip joint. The movements in this case were rapidly alternated. Each neuron of the pair is at its minimal rate of discharge when that of its fellow is maximal.

The results given in this section confirm for the monkey an observation first made in the somatic sensory cortex of the cat (33), that some neurons lying very close together in the cortex and related to one joint may be activated reciprocally by reciprocal movements of that joint. The further and very striking fact should perhaps be emphasized, that for mon-axial joints the very large majority of the related cortical cells are sensitive to movement of that

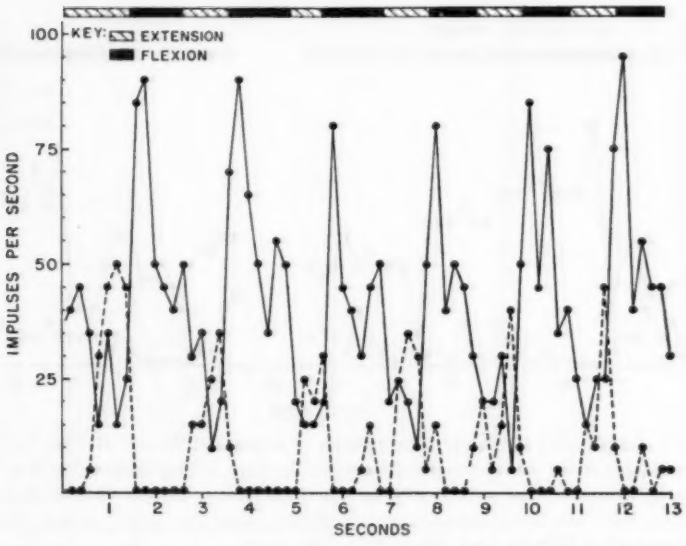

FIG. 12. Graphs plot impulse frequencies of neurons 15-7 and 15-7-a during rapidly alternating flexions and extensions of the contralateral femur upon the pelvis. Each is at its lowest when the other reaches its maximal firing rate: they display reciprocal activity patterns. Cells isolated 1810 μ below the cortical surface of area 2 in penetration M15-P7, and observed for only a few minutes.

joint in one direction only. For example, in our total of 86 neurons studied which were related to the elbow joint (considering only flexion and extension) only 4 were activated by both of two opposite movements.

III. Inhibition of Cortical Neurons Related to the Joints by Stimulation of the Skin

We have never observed in our experiments an instance of excitatory interaction between the afferent inputs from the skin and from the deep tissues which play upon cortical neurons, i.e., a neuron activated by stimulation of the skin as well as by joint movement. However, we have observed, for nine neurons, a form of inhibitory interaction, in which stimulation of the skin serves to suppress both the spontaneous and the evoked activity of a cortical cell which is excited by joint rotation. (The frequent occurrence of skin-skin inhibitory interaction is described in the following paper.) Details regarding these nine units are given in Table II. Although the number of units in which this phenomenon was observed is too small for very broad generalization, we believe the observations are valid. Six of the neurons were studied for more than one hour, and the nature of the adequate excitatory stimuli, the presence

TABLE II

Tabulation of Cortical Neurons Sensitive to Joint Rotation Which Were Inhibited by Stimulation of the Skin

Unit	Pen	Depth	Cyto Area	Joint	Excitatory Movement	Inhibitory Field—Skin of
4-14	4-2	1920 μ	1	Shoulder	Internal Rotation	Ventral Forearm
5-17	5-4	1550 μ	2	Shoulder	Retraction	Palm of Hand
5-00	5-4	1700 μ	2	Shoulder	Retraction	Palm of Hand
6-4	6-1	2390 μ	3	Shoulder	Internal Rotation	Postaxial Wrist
11-4	11-1	2580 μ	3	Elbow	Flexion	Forearm
17-3	17-1	1390 μ	—	Hip	Abduction	Ankle
24-7	24-2	2190 μ	3	Knee	Extension	Calf
24-9	24-2	2390 μ	3	Hip	Abduction	Lower Leg
24-10	24-2	2390 μ	3	Knee	Extension	Lower Leg

of inhibition, the nature of the adequate inhibitory stimuli, as well as the location and extent of the inhibitory receptive fields were repeatedly determined.

The first important fact about this phenomenon is that it is uniformly "from skin to deep". We have not observed the reverse sequence. The second fact of interest is indicated also in Table II and in Figures 13, 14, and 15: the topographical relation is such that the inhibitory receptive field is always distal—and often quite distal—to the joint whose movement drives the neuron. Four joints are represented in our small sample. An instance of this phenomenon is illustrated in Figure 13. Neuron 5-17 was isolated and found to be excited by retraction of the shoulder joint. With the shoulder held in steady retraction the neuron responded at a more or less steady rate, as shown in the chart to the upper right. When the discharge of the neuron was maintained by steady retraction, it was found that touch or light pressure to the skin of the hand produced an immediate and nearly complete cessation of the discharge, as shown in the chart below. When the inhibitory stimulus was removed, the neuron once again began to discharge in response to the steadily maintained shoulder retraction. In this instance that level of activity was somewhat less than the pre-inhibitory frequency. Commonly, however, a post-inhibitory rebound to a higher frequency is observed. That it was indeed stimulation of the skin and not deep tissues which produced this inhibition was confirmed by electrical stimulation of the skin.

The inhibition produced is not always so complete. In a somewhat similar situation, neuron 6-4 was driven by rotation of the shoulder joint and inhibited by light touches upon the skin of the postaxial wrist and forearm. However, in this case, after initial complete inhibition the neuron quickly began to discharge again at a low rate in spite of the steadily maintained light pressure upon the inhibitory skin field (chart to the upper right, Fig. 14). The experiment then was repeated using a spatially and temporally oscillating stimulus to the skin field, which produced complete inhibition (chart to lower right,

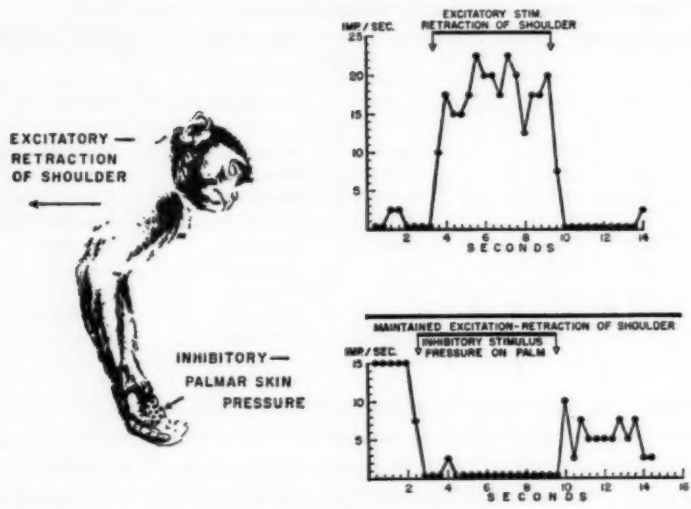

FIG. 13. Data concerning neuron 5-17, which was driven by retraction of the contralateral shoulder. Impulse frequency during such a steadily maintained retraction is shown by the graph to the right above. That activity was inhibited by light pressure upon the skin of the palm, as shown by the second graph. Cell isolated 1550 μ below the cortical surface of area 2, during penetration M-5P4, and observed for 68 minutes.

Fig. 14). That the stimulus to the skin was in fact the effective inhibitory one was demonstrated by electrical stimulation of the skin (chart to lower left, Fig. 14).

In each instance of this type of inhibition we have observed that the inhibitory skin stimulus also activated other nearby cells, and in four instances we have observed the discharges of these adjacent neurons simultaneously in the record. Neuron 4-15 was excited by internal rotation of the shoulder and inhibited by stimulation of the skin of the volar surface of the forearm, as shown by the chart to upper right of Figure 15, and the upper record of Figure 16. This inhibitory stimulus produced audible discharges in the loud speaker, and a slight downward movement of the electrode brought into the record the discharges of a second neuron which were evoked by the skin stimulus, as illustrated by the lower record of Figure 16, and the lower right hand chart of Figure 15. The excitatory receptive field for this second cell coincided with the inhibitory receptive field of the first. Gradations of the inhibitory effect have been observed, varying from a maximal effect at its center to a minimal one for stimuli delivered near the edge of the field.

Deep joint neurons which can be inhibited by skin stimulation have been seen in each of the three areas of the postcentral gyrus, with six of the nine located in area 3. The sample is too small, however, to be definitive. The loca-

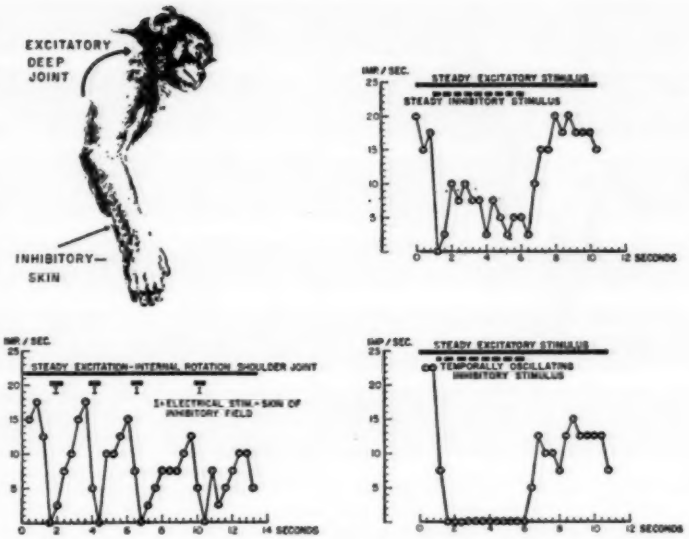

FIG. 14. Data relating to neuron 6-4, which was driven by external rotation of the contralateral shoulder joint, and inhibited by light tactile stimulation of the skin area indicated in the drawing. The graph to the right above indicates the inhibition of the steady adapted activity of this cell, produced by steady light pressure upon the skin. After the initial complete inhibition the discharge rate rose again. This is interpreted as an adaptation of the cutaneous receptors whose stimulation produced the inhibition, for an oscillating light tactile stimulation of the hairs of the peripheral inhibitory receptive field produced complete inhibition which was maintained throughout the stimulation, as shown in the graph to the right below. That the responsible receptors were in the skin is indicated by the inhibition produced by intermittent trains of electrical stimuli, delivered to the inhibitory skin field. Cell isolated 2390 μ below the cortical surface of area 1 during penetration M6-P1; observed for 110 minutes.

tions of the inhibitable neurons in relation to others studied in the same penetrations are indicated in Figure 17. Here the data for only portions of the penetrations are shown. It is clear that in each case the penetration was a mixed one. Further, in 4 of the 9 cases the deep unit which could be inhibited was isolated in the midst of a segment of the penetration in which all other neurons observed were activated from the skin. The significance of these facts is uncertain, but they suggest that this intermodal interaction occurs between cells at the region of juncture of vertical columns of neurons concerned with different submodalities of mechanoreception.

IV. Neurons Related to Deep Tissues Other Than the Joints

The data of Table I of the preceding paper show that of the more than 800 postcentral neurons studied, which were activated by stimulation of tissues other than the skin, slightly more than one-third were related to receptors in the joint capsules. The remainder were

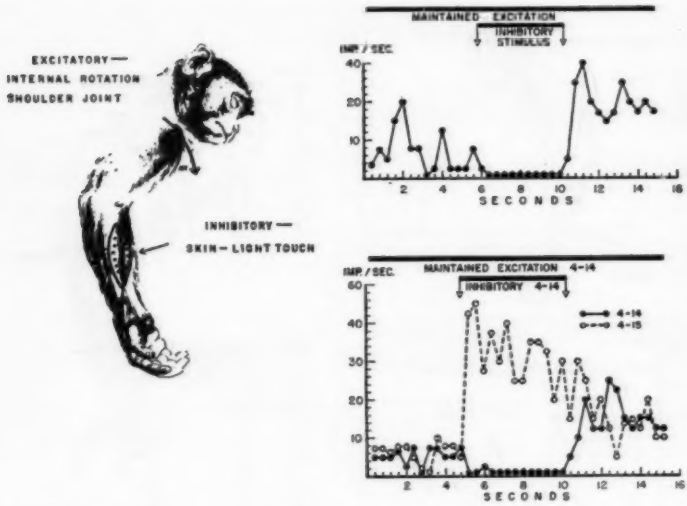

FIG. 15. Data relating to neurons 4-14 and 4-15. Neuron 4-14 was first isolated in the record; it was driven by internal rotation of the contralateral shoulder joint, and inhibited by light tactile stimulation of the skin field shown in the drawing. Impulse frequency during this excitation-inhibition sequence shown by the graph to the right above. Note post-inhibitory rebound. The inhibitory skin stimulus drove a second small unit. Slight downward movement of the electrode brought this second unit (4-15) to good amplitude, so that both were observed simultaneously. Graph to lower right plots the impulse frequencies of the two neurons during a second excitation-inhibition-excitation sequence, and shows the reciprocal patterns of activity. Cells observed 1920 μ below the cortical surface of area 1 during penetration M4-P2; observed for 50 minutes. Records from which the two graphs were made are shown in Figure 16.

responsive to mechanical stimuli delivered to the periosteum of the bones, the deep fascia enveloping muscle bellies, and—in much smaller numbers—to mechanical distortion of the superficial fascia, the connective tissues about tendons, and the deep connective tissue of the palm of the hand and the sole of the foot. The location of the receptors whose stimulation drove the cortical cells of this class was confirmed directly in a number of instances by dissection of the peripheral tissues. Such a procedure, accompanied by light mechanical stimulation of the exposed tissues, produces unmistakable evidence of the location of the receptor-bearing tissues pertinent for the neuron under observation. We have not observed cortical neurons to be driven by the stretch of muscle tissue itself.

Neurons related to the periosteum are exquisitely sensitive to mechanical stimuli. Like the neurons driven by joint movement they display a variety of adaptive rates, broadly divisible into those which continue to discharge steadily under the drive of a steady stimulus, and those which discharge an onset transient with application of the stimulus, but whose discharge rates quickly fall to the "resting or spontaneous" rate during continuance of the stimulus. The receptive fields of the neurons related to the deep fascial sheets are of sizes and distributions similar to those for neurons related to the skin.

The neurons whose receptive fields lie in deep fascia or the periosteum have extremely

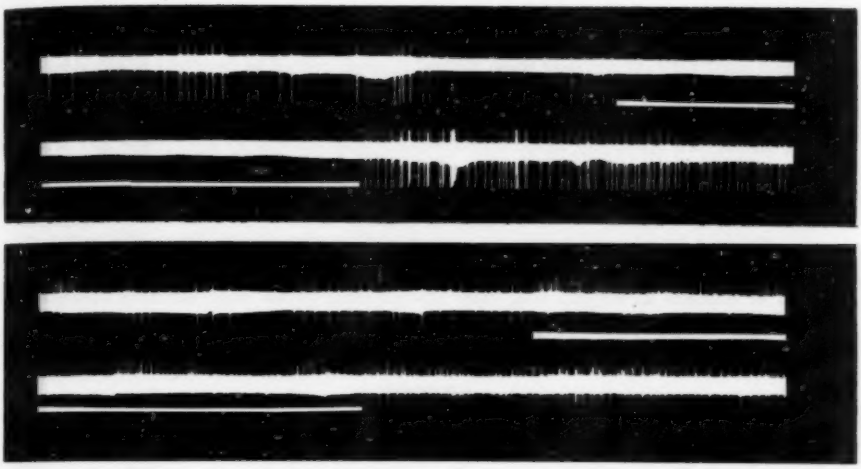

FIG. 16. Records of the impulse discharges of neurons 4-14 and 4-15. For description see the legend of Figure 15, and the text. Short time constant recording.

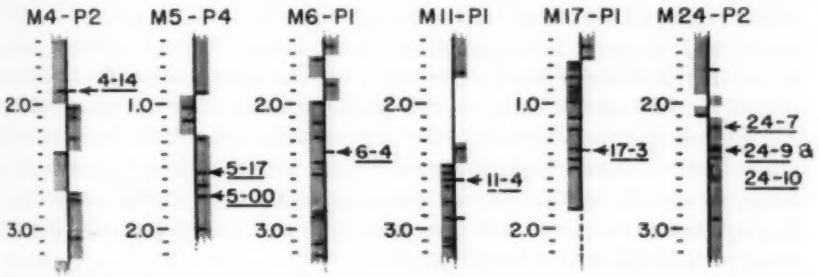

FIG. 17. Segments of reconstructions of those penetrations in which the nine neurons driven by rotation of joints and inhibited by stimulation of skin were encountered. Cross hatching and short horizontal lines to the right indicate multiunit record and isolated neurons driven from the deep tissues, respectively. Similar symbols to the left indicate the skin was the source of the afferent input driving the cortical neurons observed. Vertical scale to the left of each reconstruction indicates in 0.1 mms. the depth below the cortical surface. Note that in each instance the deep neurons that could be inhibited from the skin were encountered either (a) in the midst of a traverse across cortical tissue related to the skin: 17-3, 11-4, 6-4, 4-14; or (b) very close to a site of change over from one submode to the other: 5-17, 5-00, 24-7, 24-9, 24-10.

low thresholds to mechanical stimuli, the force of which is transmitted through the skin. We have, for example, frequently observed movement of the long and rather rigid hairs on the monkey's body to activate a cortical cell, to discover upon closer examination that the receptive field for the neuron actually lay in the fascia, and not the skin. It is because of this extraordinary sensitivity that we think the receptors of the fascia and their central

connections are components of those neural elements subserving the general sense of touch-pressure. It seems likely that all but the very lightest of mechanical stimuli of the body surface must activate not only those cortical neurons related to receptors of the skin, but those related to the superficial and deep fascia as well. Whether the receptors of the periosteum and their central connections are also concerned with the sense of touch-pressure is unknown, but their sensitivity to mechanical stimuli is almost equally great, especially at such body prominences as the elbow or knee. What role they may play in position sense or kinesthesis is unknown.

On several occasions we have observed neurons which were sensitive to mechanical stimulation of the deep tissues of the chest wall to fire rapid bursts in unison with inspiratory movement of the chest. In other instances we have seen deep fascial units to fire a short train of impulses in rhythm with each arterial pulse in the deep fascial compartment. What role this relatively high level representation of respiratory and cardiovascular events may play in the regulation of those same events is a matter for further study.

DISCUSSION

The evidence we have presented suggests that a certain class of cells of the postcentral gyrus is capable of depicting by its patterns of activity the steady angles of the joints of the body, and of transient changes in those angles; it subserves the somatic sensory components of the senses of position and movement. Tentative evidence has in fact been available for some time (34, 11, 38) which suggests that afferent fibers from peripheral receptors located in and about the joints project into the dorsal column-medial lemniscal system, relay in the ventral posterior nuclear complex of the thalamus, and finally reach the somatic sensory cortex. At each level elements devoted to this submodality are intermingled with those of others in a single and mutual somatotopic pattern (33, and unpublished observations of Mountcastle and Rose). The findings predicted by these earlier results were first made in the cat somatic sensory cortex, and have now been confirmed and extended in the present study of the monkey postcentral gyrus.

Whether cortical elements such as those described and their linked receptors and relay neurons are both necessary and sufficient for this function is a question which the experimental observations cannot yet answer finally. Assuming for the moment, however, that this is indeed the case, several important questions are present for discussion. Among these are the nature of the sensory receptors of joints, their innervation and functional properties, and the relation of their response patterns to those of cortical neurons sensitive to joint position and movement. Since our thesis is contrary to the widely held belief that position sense and kinesthesis depend, at least in part, upon the afferent input from stretch receptors, some attention will be given to the question of whether these latter are suited by their functional properties to serve this form of somatic sensibility.

(1) *The innervation of joints*

Although it has been known for a long time that the joints receive a rich innervation, this innervation and the morphology of the receptor organs of ligaments and joint capsules

have been intensively re-studied in recent years (2, 4, 9, 37, 38, 40, 41, 43). Receptor endings of the spray or Ruffini type are plentiful in joint capsules, while Golgi type endings are found in association with ligaments. Large receptors resembling Vater-Pacini corpuscles are found rarely, and they lie in the pericapsular connective tissue. No organized endings are found in synovial lining membranes. These receptors are supplied by myelinated A fibers in the size range of 2 to 16 μ, thought by some to be distributed unimodally (38), and by others bimodally (10) between these extremes. An important anatomical finding has been made by all these investigators. That is, that the afferent fibers from joint receptors may join either mixed or cutaneous nerves, or may even pass directly through muscle tissue to join the muscle nerves. Such, at any rate, are the results of studies of the knee joint of the cat. Receptors from the medial aspect of this joint are innervated by afferent fibers which pass via the medial articular nerve to join in some cases the saphenous nerve alone, in others some of these fibers enter also either or both the obturator and femoral nerves. Afferents from receptors of the posterior joint capsule of the cat's knee are collected in the posterior articular nerve, which joins the tibial nerve. Other afferent fibers from the joint pierce the substance of the sartorius muscle and enter its nerve. A similar distribution of the afferent fibers from joints to nerves commonly regarded as purely "cutaneous" or "muscular" has been confirmed for the monkey by Stilwell (39, 40, 41) and it can be considered a generality. This last investigator has also shown that the receptors of deep fasciae and aponeuroses are innervated by nerve fibers from both muscular and cutaneous nerve trunks.

These anatomical observations have been described in some detail, and we wish to emphasize them. Their implications are of great importance for experiments upon the somatic afferent system, and indeed for a very wide range of neurophysiological investigations. Their general importance is this: it is not possible to determine with any degree of certainty the tissue source of a group of afferent fibers to be activated by electrical stimulation of bared nerve trunks, by selection of this or that nerve. Furthermore, a whole host of recent investigations, which we will not review here, emphasizes a second general principle: the modality subserved by the fibers of a nerve activated by an electrical stimulus cannot be determined by measurement of conduction velocity or fiber diameter. The size spectrum of those fibers activated by a given form of stimulation may cover an extremely wide range, including the unmyelinated group (e.g., for touch, see Zotterman (44), and Douglas and Ritchie (6)). Iggo has shown very recently, by the technically brilliant feat of recording from single myelinated fibers innervating the skin, that some are selectively sensitive to mechanical stimuli, while others respond only to heat, and still others only to cold. It is clear that any given form of stimulation may activate afferent fibers of all size groups, and that modality cannot be determined by observations of the elevations of the compound action potential. These facts make it a requisite for experiments upon the somatic afferent system that physiological stimuli be used.

(2) *A comparison of the response properties of joint receptors with those of post-central neurons sensitive to joint rotation*

The response properties of joint receptors are now very well understood, thanks in the main to the clear results of the researches of Boyd and Roberts (5), Andrew and Dodt (3), and Sten Skoglund (38), using the method of single unit analysis. The experimental results obtained by these workers are supplementary to one another, and differ on no major points, and in that which follows their results will be described together. The response patterns of the first order afferents activated by joint rotation are at once divisible into two groups, those which adapt rapidly, i.e., which respond only during movement, and those which adapt slowly to a steady state frequency of discharge. This steady discharge is maintained so long as the joint is held within the excitatory angle for the unit under study. The quickly adapting elements have been tentatively correlated with the Vater-Pacini corpuscles; they occur very rarely; e.g., they accounted for only 7 of the 121 units in the population studied by Boyd and Roberts (5).

The slowly adapting receptors respond to movements into their excitatory ranges with high frequency transient discharges. If the movement comes to an end at a steady position within the excitatory range, the frequency of discharge adapts over a period of a few seconds to a lower and more or less steady rate. The remarkable and important fact about these receptors is that the level of the steady adapted rate of their discharge is a variable influenced only by the angle of the joint: *they function as absolute detectors of angle.* The adapted rate of discharge for any given angle within the excitatory range is, within rather narrow limits, the same regardless of the speed or the direction of the movement which brought the joint to that final steady angle. The discharge of a receptor with the joint in a steady position within its excitatory range varies only slightly with time, and was observed in several instances by the authors quoted to persist undiminished for several hours. Movements within the excitatory range from a more to a less excitatory position produce a reciprocal sequence—a transient decrease in the rate of discharge with recovery to a new and lower steady rate.

These joint receptors are sensitive to joint rotation over ranges of about 15–20 degrees of arc. Many show their maximal steady adapted rates at full flexion or full extension. Many others subtend angles in between, and movements of a joint such as the knee from full flexion to full extension will traverse the overlapping ranges of a successions of receptors. The profile of which receptors are active and their steady frequencies pictures the angle of the joint for any steady position. Which neurons show transient increases, and which transient decreases, in rate of discharge, and the extent of these changes will depict the directions and the speeds of movement. Both the spray type endings of the joint capsules and the Golgi tendon organs of the ligaments have been identified as slowly adapting receptors. They differ in two ways: (a) the receptors of the ligaments show much less rapid transient responses with movement, and (b) their steady adapted rates, even at maximal excitatory angles, are considerably lower than those of the capsule receptors.

While our data obtained in studies of the cortical neurons sensitive to joint rotations are less complete than those available relating to the joint receptors, the similarities between the properties of the two populations are obvious and

striking. Quickly adapting cortical neurons have been observed much less commonly (19 per cent of all) than have slowly adapting ones. The latter show rapid onset transients, with decline to steady adapted rates during steady joint positioning, just as slowly adapting receptors do. The form and duration of that decline is much more variable for cortical neurons than for joint receptors, an event conditioned in large part, we believe, by the presence of an anesthetic agent, and by the "spontaneous" ongoing activity of the cortex. The steady discharge of a cortical neuron, like that of the peripheral receptor, has been observed to persist for very long periods of time with the related joint at a steady position within the excitatory range for the neuron under study. These results indicate, as Adrian first showed (1), that the discharge properties of neurons of the somatic sensory cortex are determined by those of the peripheral receptors to which they are linked

There are, however, certain differences. The most obvious one is that the large majority of cortical neurons are sensitive to joint position in a very wide range. Their excitatory angles are of the order of 60–90 degrees of arc, rather than the 15–20 degrees of the receptors. The majority of cortical neurons, as of the receptors, show maximal steady adapted rates of discharge when the joint to which they are related is maximally displaced. The driven cortical cell in achieving increasing steady adapted rates from (e.g. for the elbow) a minimum at an angle of 90 degrees to a maximum of 180 degrees must be successively driven by the discharges of receptors whose narrower but overlapping excitatory angles are distributed over this extent of arc. If this is true it forms an example of a subtle and interesting form of integrated neural action.

(3) *Can muscle stretch receptors provide information concerning position or movement of the limb?*

If muscle stretch receptors are thought suitable to subserve position sense and kinesthesis, the question must be asked whether their functional properties allow them to perform as detectors of absolute joint angle, and of the directions and velocities of joint rotation. This would require that muscle stretch receptors supply information of muscle length, independently of muscle tension. Evidence accumulates from recent research, however, that neither of the major stretch receptors of muscle varies its frequency of discharge in a linear or even in any sort of constant relation to the length of the muscle, per se, in the active, waking animal. Since the classical work of Matthews (31) it has been known that the Golgi tendon organs discharge afferent impulses at a rate related to tension. The tension to which these receptors are subjected depends upon the length of the muscle, i.e., upon joint angle, and upon the force exerted by the active muscle against its load. It follows that the number of Golgi organs and their rates of discharge are not variables dependent solely upon the angle of the joint across which the muscle works: they cannot, therefore, inform of joint position.

The spindle organ receptors of muscle are subject to even more complex influences. Matthews had shown that these receptors are excited by stretch of muscle, but cease to discharge as the muscle is shortened by alpha motoneuron action. Under these circumstances

they may be completely silent when tension at the tendon is maximal. The work of Leksell (28) revealed, moreover, that the smaller efferent fibers of the ventral roots, the gamma motoneurons, produce upon activation an increase in spindle organ activity, even when the muscle shortens. These observations have been extended by a number of workers (7, 8, 14, 15, 16, 17, 21, 22, 23, 24), and it is now well known that the gamma efferents condition afferent input from the spindles, and thus play an important role in reflex regulation, postural adjustments, and in voluntary movement. The important point in the present consideration is that spindle organ activity may vary over a very wide range of frequency independently of the length of the muscle. These receptors, like the tendon organs, are little suited to inform of muscle length, and hence of joint angle. The possibility does exist that if some central analyzing mechanism received information of muscle tension, and of the frequencies of discharge in both alpha and gamma motoneurons it could deduce muscle length by a process of some complex integration. There is no evidence, however, that impulses in gamma motoneurons are fed back centrally, for example by axon collaterals. That muscle contraction may play a minor role in the perception of joint angle is suggested by the fact that the discharge rate of slowly adapting receptors of the joint capsules is affected by the contraction of muscles which tense the joint capsules (38). And Provins (36) has recently shown that the threshold for the perception of passive movement of the joints of the human figures is but insignificantly lower when the movement is made against an active contraction.

These facts in themselves provide a powerful argument that the stretch receptors of muscle are not likely to provide information useful to the perception of joint position. They are complemented by the observations of Lloyd and McIntyre (29) that the large stretch afferents from muscle do not project upwards in the dorsal columns, but relay in the column of Clarke-Stilling into ascending systems terminating in the cerebellum. These observations have been confirmed and extended in an elegant series of single unit analysis studies which have shown that group Ia and Ib afferents from muscle relay into the dorsal spinocerebellar tract, and group Ib from tendon organs upon the cells of origin of the ventral spinocerebellar tract (20, 25, 26, 27, 30, 35). Complementary also are the negative observations that afferent volleys confined to group I afferent fibers from muscle evoke no response in the somatic sensory cortex (34). In this regard, it is probably only these group I fibers which can be assigned to particular sets of peripheral receptors. Nor have any studies, including the present ones, revealed any cells at thalamic or cortical levels responsive to stretch of muscle (33, and unpublished observations of Mountcastle and Rose). Some differences of opinion exist as to whether group II and group III or only group III myelinated afferents from muscle do project upon the somatic sensory cortex (32, 34). In view of the clear projection of joint afferents into some muscle nerves, these differences now seem readily explicable.

(4) *Correlations with observations in humans*

From the time of the classic studies of Goldscheider (12, 13) evidence has been available that the human perception of joint position depends upon afferents arising in the joint receptors. His careful work and his clear recognition of the joints as the source of kinesthetic information fits well with clinical observations of humans with denervated joints (e.g. 42). It fits also with modern psychophysical work using quantitative measures of joint angle and movement perceived, combined with local anesthesia of joints and pericapsular tissues. We will not review this large subject in any detail, but wish to cite as an ex-

ample the studies of Provins (36), who found a highly significant impairment of the threshold for the perception of movement of the human finger when the region of the metacarpo-phalangeal joint was locally anesthetized, without affecting the innervation of muscles acting at that joint. Concerning the possible role of muscle stretch afferents in position sense and kinesthesis it is important to note the observation that humans with lesions of the cerebellum show no defects in these forms of sensibility (18, 19).

(5) *Population events in the postcentral gyrus set in motion by joint rotation*

All of our observations, obtained upon a large number of cortical neurons sensitive to joint movement, lend themselves to an imaginary reconstruction of the events occurring in the cortex during bodily movement, and during steadily maintained attitudes. The afferent cortical input derived peripherally from joint receptors will fractionate the group of cells related to a given joint. At any steady position some neurons will discharge rapidly, in the steady adapted state, others will discharge less rapidly in the same state, while still others will be active at a normal "resting or spontaneous" rate. Our observations suggest that some other cells will be inhibited, either in part or completely, in a reciprocal manner to those excited, for closely adjacent pairs of cells have been observed frequently which respond in a reciprocal manner to movement of the same single joint. The projection pattern of the body form determines the *place* of the activated and inhibited cells in the cortex. Thus a spatial and temporal pattern of activity in a considerable population of neurons will exist which is unique, or nearly so, for that particular joint angle. As the joint moves away from that steady position the spatial and temporal profile of activity will abruptly change. Some neurons, previously silent, will discharge short, high frequency bursts of impulses only during the movement, and may subtend narrow angles. Other neurons, previously silent, will also discharge high frequency movement transients, will subtend wider excitatory angles, and will continue to discharge at a steady rate with the limb in the new position. Still other neurons, active at one rate or another in the old position, will assume new and lower levels of discharge, or in fact may be rendered completely silent by virtue of reciprocal afferent inhibition. This shifting panorama of cortical activity depicts the peripheral event—that a movement has occurred or a new postural attitude assumed. No inkling is yet forthcoming from studies upon the cerebral cortex concerning that necessary cortical mechanism for recording and interpreting this picture of the peripheral state of affairs, that is, of the neural process interpolated between these primary and simple cortical events set in motion by an impinging peripheral stimulus, and the percept evoked by it.

One general principle is supported by these observations, namely that the central nervous system utilizes *both* the place of occurrence of neural activity

and the temporal pattern of neuronal discharge to depict the place, intensity, and temporal cadence of sensory events.

SUMMARY

A description is given of the functional properties of a class of neurons of the postcentral gyrus which is sensitive to movements and steady angles of the joints. Such a neuron discharges rapid onset transients when the joint to which it is related is moved in the excitatory direction for the cell. The large majority thereafter discharge at steady rates with steady joint positions within the excitatory range of joint angle. The level of that steady rate is determined by joint angle, and is independent of the speed or direction of the movement bringing the joint to that angle. Movement of a joint away from the excitatory direction causes a decrease in the discharge rate of such a cortical cell, and closely adjacent cells responding in a reciprocal manner—the one excited and the other inhibited—to a given movement have been observed. Evidence is presented to support the hypothesis that this class of cells of the postcentral gyrus, together with the relevant peripheral receptors, afferent nerve fibers, and relay neurons, constitutes the essential neural substratum serving the somatic sensory component of the senses of position and kinesthesis.

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NEURAL MECHANISMS SUBSERVING CUTANEOUS SENSIBILITY, WITH SPECIAL REFERENCE TO THE ROLE OF AFFERENT INHIBITION IN SENSORY PERCEPTION AND DISCRIMINATION[1]

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INTRODUCTION

In this paper we wish to describe the properties of those cells of the postcentral gyrus of the monkey which respond to light mechanical stimuli delivered to the skin. They make up, at least in part, the neural substratum of that mode of cutaneous sensibility commonly referred to as touch-pressure. That they alone are concerned in the cortical mechanisms involved in touch-pressure is very unlikely, for our observations described in the preceding paper (16) indicate that very large numbers of cortical neurons are related to receptors contained in fascia, connective tissues, and the periosteum. All but the very lightest of stimuli must bring to action large numbers of this last class, and it seems likely that the two groups must be considered together as intimately involved in the cortical mechanisms of mechanoreceptor sensibility.

The first parts of this paper describe the response properties of cortical cells excited by cutaneous stimuli. These are not presented in great detail, however, for they have but confirmed many of the earlier observations made in the first somatic sensory cortex of the cat, the postcentral homologue in that animal. (13, 14). In a later part the results of our studies of afferent inhibition are given, and stress is laid upon the importance this property of the somatic afferent system appears to play in sensory discrimination. Finally, we give the results of studies of a few neurons which differ in many respects from those of the major population studied. Their properties resemble in almost every way those of neurons of the posterior thalamic group (18), and indicate, we believe, a projection of two quite different components of the somatic afferent system upon the postcentral gyrus.

METHODS

The data presented in this paper were obtained from studies of 593 neurons of the postcentral gyrus of the macaque monkey. These form a part of the total population studied,

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and some of their properties have already been used in a formulation of the functional organization of the postcentral gyrus (19). The spontaneous and evoked activities of cortical neurons were recorded by means of microelectrodes, from an extracellular position. The electrodes were passed into the cortex, via small pia-arachnoid openings through a fluid tight chamber, by means of a Davies microdrive (6). The method has been described in detail in the second paper of this group (19), and in an earlier report (14); the former presents in some detail the methods used in detailed studies of the serial sections of the brains, and locations of electrode tracks.

That certain neurons of the somatic cortex are sensitive only to mechanical distortion of the skin can readily be shown by surgical elevation of the skin, its innervation preserved intact; under these circumstances one finds the neurons concerned responding in the same way to stimulation of the elevated skin, and insensitive to stimuli delivered to the subjacent deep tissues. In general the sensitive relation of the evoked discharge of such a cell to very light mechanical stimulation of the skin, and the unvarying position of its receptive field when the skin is displaced or elevated into folds, are convincing evidence of the cutaneous location of the receptors concerned. We believe that this identification can be made very accurately if great care is taken in these maneuvers. Electrical stimulation of the skin is a convenient way of confirming the cutaneous origin of the excitatory afferent impulses.

The locations and extents of cutaneous receptive fields were determined using light mechanical stimuli, the outlines marked on the skin, and photographed. Variations of the response of a cortical neuron produced by variations in the location, intensity, and frequency of stimulation were studied using short (0.1 msec.) electrical stimuli delivered through a fine pair of electrodes thrust into the skin. We believe such stimuli activate the fine nerve filaments within the skin, and not their endings. Unpublished observations obtained with intra-axonal recording from single fibers of the dorsal columns, driven by such cutaneous stimuli, indicate that a stimulus duration of about 0.75 msec. is required to produce more than one impulse per afferent fiber. Thus the repetitive firing of cells of the central regions of the somatic afferent system to single stimuli is not uniquely dependent upon repetitive input in the same single first order afferent fibers. We have attempted whenever possible to compare the results obtained by using electrical stimulation of the skin with those produced by physiological stimuli. While these latter are not subject to careful quantitative control, it can be said that no qualitative differences in the nature of the responses have been observed. Adaptive properties were tested by applying steady mechanical stimuli to the skin. The action potentials of neurons active under such steady drive were counted in each short interval (0.1, 0.2, or 0.4 second) and plotted as running averages of impulses per second. This gives a much more accurate estimate of the overall level of activity than one obtains by plotting the reciprocals of intervals.

RESULTS

I. Topographic Attributes of Postcentral Neurons

The first observation one makes upon postcentral neurons which are related to the skin is that each may be activated by light mechanical stimulation of a certain sharply delimited area of the skin. Each cell has a peripheral receptive field, and stimulation elsewhere does not excite it. The second observation is that the sizes of these receptive fields vary with the positions of the fields upon the body surface. The areas of the fields of 195 postcentral neurons sensitive to the stimulation of the skin of the contralateral hand and arm are indicated in

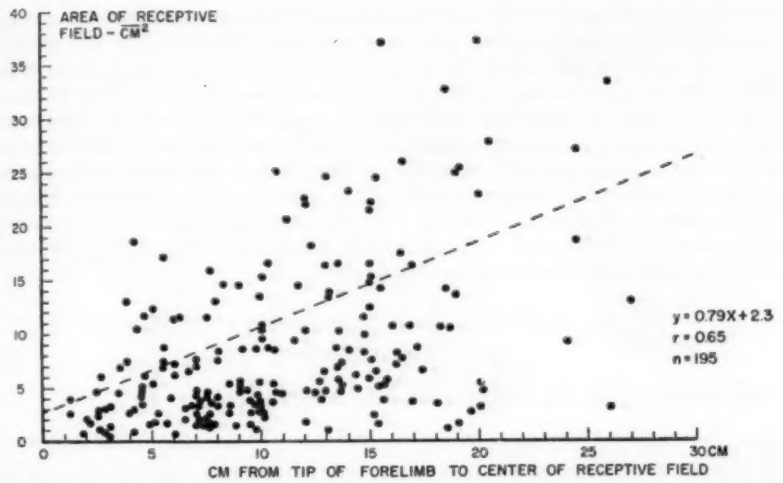

FIG. 1. Graph upon which each black dot relates the area in cm.² of the excitatory peripheral receptive field for each of 195 cortical neurons to the distance from the center of that field to the finger tips. All fields located on the contralateral arm. The population includes cells located in each of the three cytoarchitectural areas of the postcentral gyrus.

Figure 1. It will be seen there that a fairly good correlation exists between the size of the field and its distance from the tip of the limb ($r = 0.65$). The pooled population of Figure 1 contains data for neurons located in each of the three cytoarchitectural areas of the postcentral gyrus. When neurons of areas 3 and 1 are grouped separately and compared, no significant differences between them appear in relation to this property. So few neurons of area 2 were found to be driven from the skin (see 19) that no certain statement can be made regarding the areas of the cutaneous fields which play upon them.

It has been shown by means of mapping experiments that the volume of tissue of the thalamic relay nuclei (15) and of the postcentral gyrus (27) which is devoted to a given peripheral part is directly related to the density of the innervation of that part. That is, it is not the form of the part which is represented as such in the central nervous system, but the pattern and density of its innervation. Our present results indicate the reciprocal of this principle; the denser the innervation of a part the smaller the cutaneous fields which play upon the cortical neurons related to the part. Nevertheless it is clear from our present studies in the monkey, as well as from earlier ones in the cat (13), that the capacity of this system for form and contour recognition far exceeds that predicted by a simple anatomical point to point relation of the periphery and the neural centers. The relation is clearly point to area and area to point. More dynamic physiological events must be invoked to explain the capacity of the system. This matter will be the subject of a later discussion.

The relations of the receptive fields of cortical neurons to the overall patterns of projection of the body form upon the postcentral gyrus have been discussed in some detail in the second paper of this series (19), and will not be repeated here. Suffice it to say that all of our topographic studies of cortical neurons conform to the original observations of Marshall, Woolsey, and Bard (27), which suggested that the periphery is represented in terms of dermatomes or segments, each occupying an anteroposterior strip of postcentral gyrus extending across areas 3, 1 and 2. These areas, of course, fuse smoothly in the mediolateral direction, and taken together compose the distorted cortical image of the body form. Reference is made at this juncture to some earlier discussion (19) devoted to the observation that cortical neurons related to cutaneous or to deeply placed receptors are differentially distributed across the antero-posterior axis of the postcentral gyrus.

II. The Projection of the Receptive Fields of the Skin upon Cortical Neurons

(1) *The stability of the size and position of the receptive fields*

Once the impulse discharges of a cortical neuron of the cutaneous subgroup have been isolated from those of its neighbors, it is a simple matter to study its receptive field. The field is determined by delivering light mechanical stimuli (with a blunt rod, or air jet) to the skin, while observing the impulse discharges evoked. Studies of the receptive fields of the skin which play upon neurons of the cortex were made earlier in the first somatic sensory area of the cat (13), and those results have been confirmed and extended in our present studies of neurons of the postcentral gyrus of the monkey. The shape, size, and position of the field for each of 76 such neurons is illustrated in Figures 10 and 11 of the second paper of this series (19), in which these data were used to show the very close proximity of the receptive fields of neurons which occupy the same vertical column of cells oriented parallel to the vertical axis of the cortex.

It is important to say that we have never observed the receptive field of a cortical neuron to change its position upon the body surface. Nor have we observed its size to change with variations in anesthetic depth, and the anesthetic level has often been radically changed while a single neuron was under observation, by further intravenous injections of barbiturate. There is one circumstance, however, which will cause the boundary of the receptive field to shrink slightly (from a few mm. up to 1 cm.). That is, the inversion of the initial sign of the action potential of the cell under study from negative to positive.

This change of initial sign, and its effect upon the excitability of the cell (i.e., its drivability by peripheral stimuli), has been described in an earlier paper (14). It was shown there that when the electrode is in such a spatial relation to a cortical cell as to record its action potential with initial phase positive, the neuron will frequently show signs of a progressively more severe cellular injury. The early phase of this injury is manifested by a reduction in the number of impulses evoked by each supramaximal stimulus delivered to the center of the cutaneous receptive field. Under these circumstances the threshold of the cell is raised, so that a tenuous synaptic impingement upon it, such as that activated by

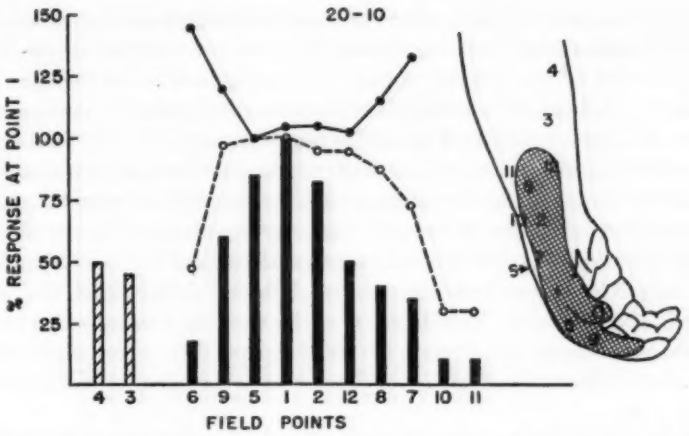

FIG. 2. Results of an experiment which illustrates gradations in the intensity of projection of a peripheral receptive field upon a cortical neuron of the postcentral gyrus. Light tactile stimulation anywhere within the area crosshatched on the inset drawing evoked responses of this cell. At each of the points marked by numerals the skin was stimulated with supramaximal electrical stimuli, delivered via needle electrodes thrust into the skin. For each point 50 responses were observed. The bar graph indicates the mean value of the impulses evoked per stimulus for each point, plotted as per cent of the mean response of the point 1 population. Stimuli delivered to points 3 and 4, outside the field determined by physiological stimulation, also evoked responses. The points were thought to lie over the nerve fibers innervating this field. Dashed and solid lines plot, respectively, the probability that a response will occur and the mean latency of the population of responses, expressed as percentages of these values for the response populations obtained during stimulation of point 1. Cell isolated 2000 μ below the cortical surface, in area 3, during penetration M10-P1; observed for 110 minutes.

stimuli delivered near the edge of the receptive field, is no longer capable of bringing the neuron to action. Many such neurons are so severely damaged that they can no longer be driven at all by peripheral stimuli. The later and more severe stage of damage characterized by rippling epilepic discharges is more obvious, and better known.

(2) *Gradients within a receptive field of its capacity to excite a cortical neuron*

A common observation is that all parts of its cutaneous receptive field do not project upon a cortical neuron with the same potency for excitation. An example is illustrated in Figure 2, neuron 20-10, which was located in area 3. The receptive field determined using physiological stimuli is shown in the drawing; it covered the preaxial wrist and the thumb and first finger of the contralateral hand. The gradient across this field of its capacity, upon stimulation, to excite the cell is indicated by the bar graph. At each point marked on the figure (the points are numbered in the order examined) a pair of small electrodes was thrust into the skin. Using supramaximal stimuli delivered at a rate of 20 per

minute, 50 responses of the neuron were recorded for each point stimulated. The bar graph indicates the mean number of impulses per stimulus as a percentage of that evoked by the stimulus at point 1. That the number of impulses per response of a thalamic or cortical neuron is a sensitive index of its degree of excitation has been established in earlier investigations (21, 14). One curve of Figure 2 indicates the mean latency of the population of responses for each point; the latency lengthens as the stimulus moves towards the edge of the field. Another curve plots the probability that any response will occur at all. Although the probability of response decreases as the edge of the field is approached, this change is a poor indicator of the differing capacities of the various points in the field, when stimulated, to excite the cell. The use of this method and the use of modal distribution curves of a population of responses of a cortical cell as indices of its degree of excitation have already been described in detail (21, 14).

Such a uniform gradation as that described is not, however, observed for all neurons. In many instances the most effective site for stimulation is excentrically placed within the peripheral receptive field. Another variant, rarely seen, is illustrated in Figure 3. This neuron was also located in area 3, and its long narrow receptive field occupied the postaxial border of the contralateral lower forearm and wrist, extending symetrically onto both dorsal and volar surfaces; only the dorsal is shown in the figure. Populations of responses were collected for each of several points along the proximal-distal axis of the field. The bar-

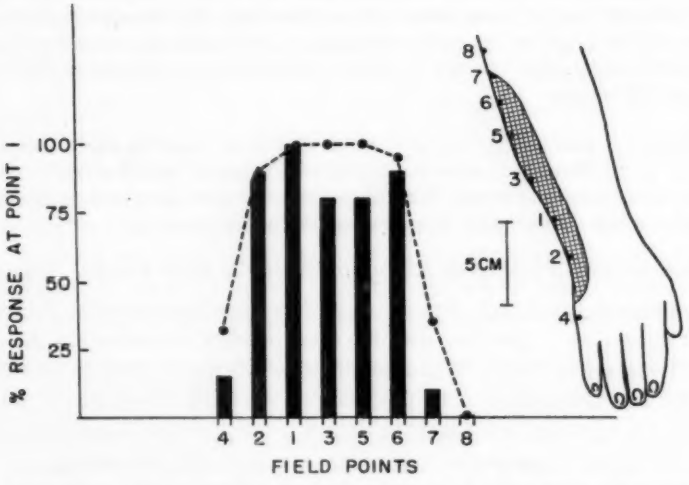

FIG. 3. Study of projection of the peripheral field upon neuron 27-4. For description of study, see legend of Figure 2. Bar graph and probability of response curve show a nearly even intensity of projection across the field, and precipitous drops at its edges.

graph of Figure 3 shows that the intensity of the projection of this field upon the cortical neuron was very nearly uniform over a very great distance, dropping precipitously near its edges.

In summary, the peripheral receptive fields of cortical neurons excited by stimulation of the skin are stable in size and position. Gradations in the capacity of the receptors of the skin, when stimulated, to excite the cortical neuron do exist. They are usually oriented from a maximum at the center of the field to a minimum at its edge, though eccentric orientations are frequently observed. These gradations are expressed but poorly as variations in the probability that any response at all will be evoked, and much more sensitively by changes in the degree of the response (the number of impulses).

III. Relation of the Response of Cortical Neurons to the Parameters of the Peripheral Stimuli Which Drive Them

(1) *Variations of response with stimulus intensity*

Earlier studies in the cat have shown that the probability, latency, and degree of the response of cells of the thalamic relay nuclei (21), and of the first somatic sensory cortex (14), are sensitively related to the strength of the cutaneous stimulus. The latter two changes are not dependently interrelated. Our studies of postcentral neurons in the monkey conform in every way to those made in the cat, and we will not describe them in great detail. The results of a typical study are shown in Figure 4. The distributions of the populations of responses for each stimulus intensity employed, shown to the left, indicate that with increasing stimulus intensity there is a gradual shift of the populations in the direction of an increased number of impulses per response. The mean latency figures indicate that there is a shortening of latency with increasingly strong stimuli. Such at least is the common happening, which is illustrated for this and two other units by the three upper graphs of Figure 5, in which these measurements are plotted as per cent of the values obtained with the strongest stimuli used. The probability that a response will occur at all is plotted by the third curve on each graph. It is at once obvious that this value reaches 1.0 with very weak stimuli, and thereafter gives no measure whatsoever of the further and very significant changes in the response produced by stronger stimuli. The pattern of changes shown for these three neurons, while commonly observed for others, is by no means ubiquitous. The curves for neuron 17-6 illustrate an instance in which all changes occurred over a very limited range of stimulus strengths, although the mean value of impulses per response increased over that narrow range from 0.9 to 2.2. The curves for neurons 17-1 and 4-11 illustrate the fact that changes in latency and the degree of response may vary independently of each other. This matter has been treated in some detail elsewhere (21) for thalamic neurons.

In all we have carried out intensity studies upon 36 neurons, and 28 of these

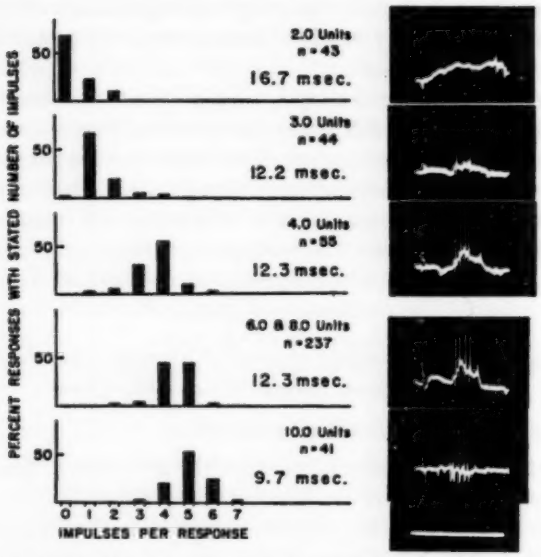

FIG. 4. Study of the relation of the response of neuron 13-14 to the strength of electrical stimuli delivered to the center of its cutaneous receptive field on dorsal surface of the contralateral forearm. Stimulus intensity in arbitrary units, the number, and the mean latency for each population given in the center column of figures.

Distributions of populations with respect to impulses per response indicated by bar graphs to the left. Long time constant recording for all except the lowest record. Time line: total duration is 40 msec. Cell isolated 2490 μ below cortical surface, in area 3; observed for 200 minutes.

studies were sufficiently complete to allow detailed analysis. Twelve were made of cells located in area 3, 10 in area 1, 3 were located in area 2, while 3 were studied in penetrations which were not found in the serial sections. So far as such a sample is valid, it is possible to say that the patterns of change in the response of a postcentral neuron produced by stimuli of changing intensity do not differ between cells of different cytoarchitectural areas.

The data presented here and in the preceding section indicate that the latency and degree of the response of a cortical cell are functions of both the *position of the stimulus* within the peripheral receptive field of the cell and of the *stimulus intensity*, and, as will be shown below, upon its immediate history as well. It is clear then that no evidence exists to indicate that *space* i.e., the position of the stimulus within the receptive field, is coded in terms of the temporal characteristics of the response (23). A further point should be made, that the *latency* of any given response is not a datum which exists for the central nervous system; its determination is a convenient neurophysiological exercise, often of great value

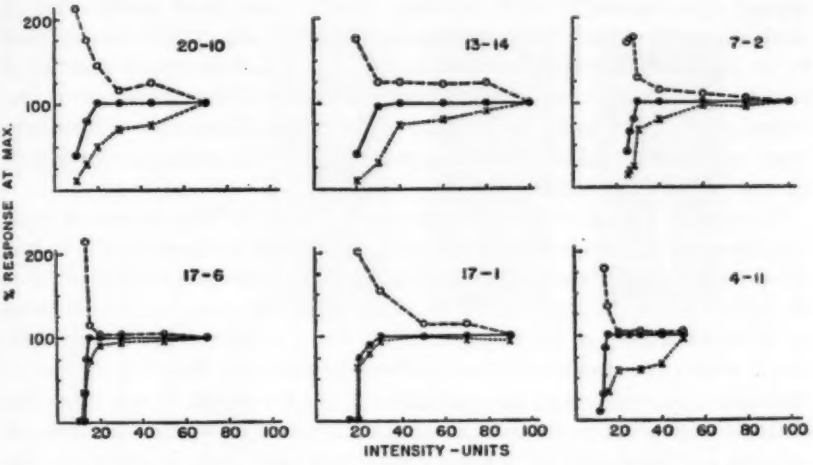

FIG. 5. Graphs of data obtained in intensity series carried out upon 6 postcentral neurons. Values are plotted as per cent of those for responses obtained at strongest stimulation level. Open circles and dashed lines—mean latencies; crosses and dashed line—mean number of impulses per response; solid circles and full lines—the probability that a response will occur.

in experimental analysis. Central mechanisms, however, can only assay the temporal relation which exists between the impulse discharges of elements of a population. Whether such a mechanism exists, and if so what its nature may be, is unknown.

It should be made clear that these data were obtained using short electrical stimuli delivered through electrodes thrust into the skin, and thus the responses are abnormal to the degree that the synchronization of afferent impulses is not a usual result of normal tactile stimuli. All of our observations made using light tactile stimuli are, nevertheless, qualitatively similar to those described above, but only limited quantitative studies using this latter form of stimulation have been carried out.

(2) *Variation of response with stimulus frequency*

(a) *Responses to the second of two stimuli delivered at various intervals.* Of all the properties of postcentral neurons which we have observed, the one which is most sensitively related to changes in the anesthetic state is the recovery of excitability following a response to a single peripheral stimulus. This relation was first analyzed by Marshall, Woolsey and Bard (12), and subsequently studied by others (see Forbes, *et al*, (7); Jarcho, (9)). All these investigators observed variations in the recovery cycle of the slow wave evoked response, recorded from the pia-arachnoid surface of the cortex with gross electrodes, to

depend upon anesthetic level. Marshall, Woolsey and Bard showed that at moderate levels of barbiturate anesthesia the excitability cycle is characterized by an absolutely unresponsive period of 10–30 msec., followed by a period of relative unresponsiveness of much longer duration. These periods were prolonged, often very greatly, at deeper anesthetic levels. These observations have been confirmed in studies of single neurons of the somatic sensory cortex in both the cat (14) and the monkey.

Three examples are shown by the graphs of Figure 6. With increasing intervals between conditioning and testing stimuli delivered to the skin, neuron 4-13 showed a gradual increase in the probability that any response would occur at all, an increase in the degree of the response when it did occur, and a shortening of the mean latency of the response. These last two approached the unconditioned values at a stimulus interval of about 70 msec., the first at much shorter intervals. Two variants of the excitability cycle are shown by the other two charts of Figure 6. The curves for neurons 7-2 and 2-10 show that the increase in response and the shortening of latency may occur along quite independent time courses.

A further and significant observation was made by Marshall, Woolsey and Bard. They observed that in the very lightly (recovering from ether) or unanesthetized (implanted electrodes) monkey an unresponsive period may not occur at all, as measured by the slow wave evoked potential. In fact, they observed in some instances a period of summation, or facilitation, during the first 20 msec. after delivery of the initial stimulus. We have observed a similar absence of unresponsiveness and an early period of facilitation for many cortical neurons subjected to the two-stimulus experiment. It is important to state that in these experiments stimuli of supramaximal strength were used, and delivered to the centers of the peripheral receptive fields.

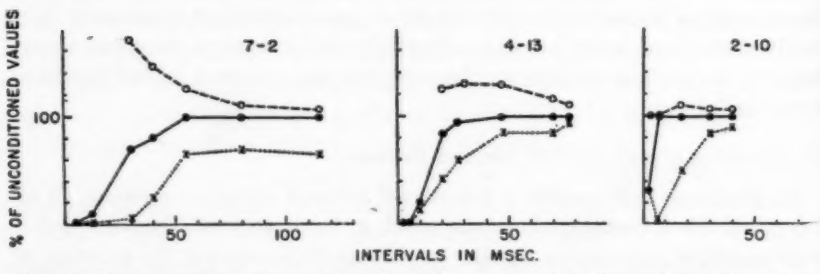

FIG. 6. Graphs plotting data obtained in studies of the excitability cycles of three postcentral neurons, relating to the response to the second of two strong and equal electrical stimuli delivered to centers of peripheral receptive fields. Open circles and dashed lines—mean latencies; crosses and dashed lines—the mean number of impulses per response for the response to the second stimulus delivered at a series of intervals, after the first. Values are given as per cent of those for unconditioned responses. Solid circles and full lines plot the probability that any response at all will occur to the second stimulus.

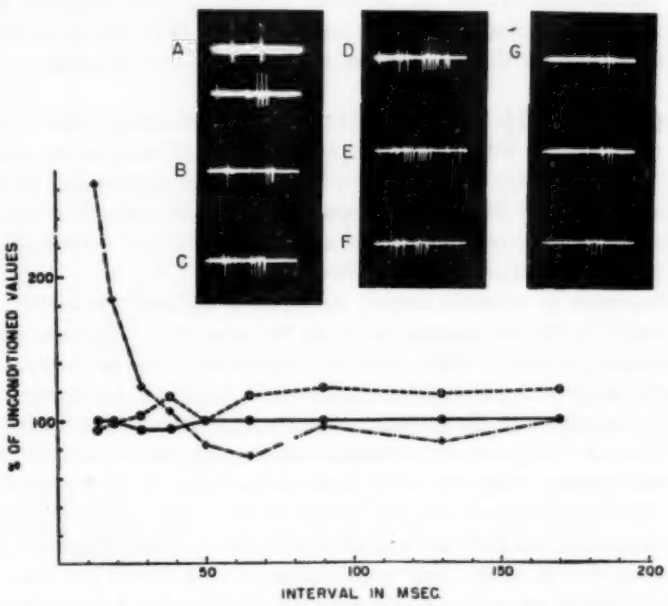

FIG. 7. Results of excitability cycle study of neuron 3-11, carried out under very light anesthesia. For method see legend of Figure 6, and the text. Plus marks and dashed lines plot the mean number of impulses per response, and open circles and dashed lines the mean latencies of the responses, to the second stimulus delivered at a series of intervals after the first, as per cent of the unconditioned response to the second stimulus. The full line plots the probability that any response will occur at all. Inset records: A—at 39 msec. interval, response to second stimulus shown on faster time base on second beam of oscilloscope; B—at 27 msec.; C—at 19 msec.; D—at 13 msec.; E—at 8 msec.; F—at 5 msec.; G—control responses to unconditioned second stimulus: mean value 2.79 impulses per response.

The results of such a study as that referred to are shown by the graphs and sample records of Figure 7; this excitability cycle was done using two strong and equal stimuli, in an animal very lightly anesthetized. The degree of response (the mean number of impulses per response) evoked by the second stimulus falls from nearly 300 per cent to 100 per cent of the unconditioned value between 13 msec. and 40 msec. test intervals. At the shorter intervals tested the trains of impulses evoked by the two stimuli became continuous, and could not be separately identified (see Fig. 7). In this very light anesthetic state very little variation of the mean latency from its unconditioned value occurred at any interval of the series. A somewhat similar excitability cycle of neurons of the somatic sensory cortex of the cat when driven by stimuli delivered to the ventral posterior thalamic nucleus has been described by Li, Cullen and Jasper (11). Li (10) has also observed that stimulation of the centre-median nucleus

of the thalamus facilitates the response of cortical cells activated by such ventral posterior nuclear stimuli. He postulates that this effect is mediated via the so-called diffuse thalamo-cortical system. We have no evidence in this regard.

It appears reasonable to assume that the behavior of cortical cells as observed in the most lightly, rather than deeply, anesthetized state more closely resembles the normal state of affairs. These observations suggest that in the waking animal the factor limiting the responsiveness of the somatic system is the frequency of response of receptors and afferent nerve fibers. Some implications of this conclusion will be discussed below.

(b) *Responses to repetitive stimuli, delivered at different frequencies.* In the earlier study in the cat referred to above the capacity of the neurons of the somatic sensory cortex to follow repetitive stimuli delivered to the skin of their peripheral receptive fields was found to be a function of the anesthetic state. At very light anesthetic levels such neurons follow the stimuli beat for beat up to 50–100/second. At higher rates of stimulation the response commonly comes to an overall average response rate. Such studies have been repeated in the monkey for 17 postcentral neurons. Sixteen of these showed the typical equilibration described; one instance is illustrated by the graphs of Figure 8. Eight of the neurons studied in this way were located in area 1, and 7 in area 3. Two were studied in penetrations which were not found in the serial sections. The only difference we have observed between the two groups is that those of area 3 tend to equilibrate at higher frequency levels than do those of area 1, a measure, we believe, of the very dense thalamocortical projection upon area 3, as compared with area 1 (3, 25, 17). It is an important point that during the period of the equilibration the response of the neuron occurs in a definite relation to certain stimuli of the train, and not to others. While stimuli which evoke responses and those which do not are randomly intermingled, the evoked discharges are not at all randomly placed. This observation has been made also by Towe and Amassian (23).

Many of the experiments described earlier in the cat (14) were done under deep Na pentobarbital anesthesia. In that condition many cortical neurons display "cut-off" frequency response curves. Such neurons follow slowly repeated stimuli faithfully. When more rapid trains are delivered these neurons respond to the first few stimuli, then cease to respond, and remain silent (or nearly so) throughout the duration of the train. We have observed only one instance of this type of behavior in the monkey, when stimuli were delivered at or near the center of the cutaneous receptive fields. The results of studies of this exceptional neuron are shown by the graphs of Figure 9; it is perhaps significant that the data upon which those curves are based were obtained shortly after the intravenous injection of a larger than usual sustaining amount of Na thiopental.

Some earlier observations had suggested that the position of the stimulus

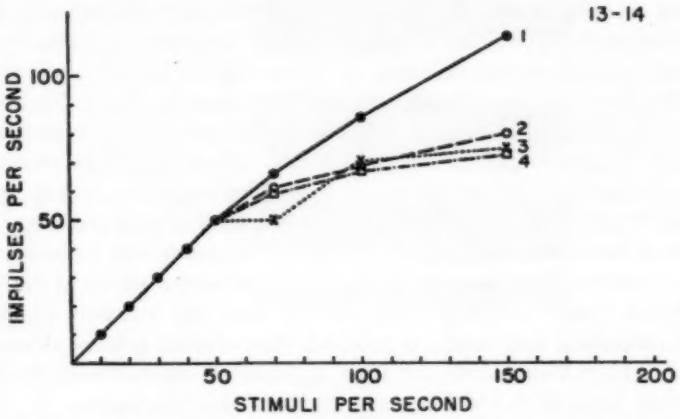

FIG. 8. Results of a frequency series study of neuron 13-14, showing typical equilibration type response. Stimuli were of supramaximal strength delivered to the center of the peripheral receptive field. Graph plots frequency of responses versus frequency of stimuli for 1st, 2nd, 3rd, and 4th seconds of each long repetitive train of stimuli. Study carried out in very lightly anesthetized state. Cell isolated 2490 μ below cortical surface, in area 3; observed for 200 minutes.

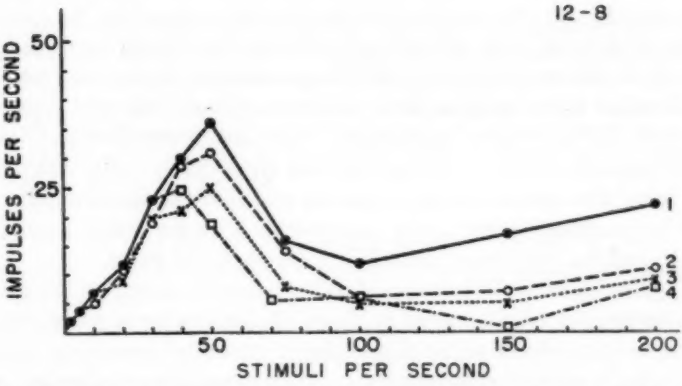

FIG. 9. Results of a frequency series study of neuron 12-8 carried out in deeply anesthetized state, showing partial "cut-off" frequency response curve. Stimuli were of supramaximal strength, delivered to the center of the peripheral receptive field. Graph plots frequency of response versus frequency of stimulation for 1st, 2nd, 3rd, and 4th seconds of each long repetitive train of stimuli. Cell isolated 3500 μ below the cortical surface, in area 3, in penetration M12-P5; observed for 50 minutes.

within the peripheral receptive field was also a critical factor in determining the frequency response characteristics of neurons of the somatic sensory cortex. This has now been made the subject of a special investigation in this laboratory by B. Z. Renkin. Her results indicate unequivocally that, under more or less

constant anesthetic conditions, a cortical neuron may display equilibrating type frequency response behavior, or "cut-off" behavior, to trains of stimuli delivered respectively to the center or to the edge of its cutaneous receptive field. We have confirmed this observation in the monkey. The intimate mechanism of this failure of response is unknown, but it is evident that the two states in which it appears have a certain similarity. Deep anesthesia renders even dense synaptic projections less secure is evoking responses than is normally the case. Further, the synaptic capacity of that limited impingement activated by stimuli delivered to the edge of a peripheral receptive field has always a low factor of safety. These patterns of response have been observed at the cortical level by Li, Cullen and Jasper (11), and by Towe and Amassian as well (23), and at subcortical relay nuclei of this and other afferent systems (Vernier and Galambos (24); Mountcastle and Rose, unpublished observations). What they may imply for sensation will be the subject of a later discussion.

(3) *The pattern of response of postcentral neurons to steady stimuli: adaptive properties*

Cells of the postcentral gyrus which are excited by stimulation of the skin differ in their patterns of response to steady stimuli delivered to their receptive fields. Of the 593 'cutaneous' neurons studied, 437 adapted quickly to steady stimuli, and 153 continued to discharge throughout the duration of the stimulus at a steady rate; this property was not determined for three cells. Of this total population, 408 were isolated in penetrations the tracks of which were later identified in the serial sections. The proportions of the cells of one or the other type did not differ significantly from one cytoarchitectural area to another (areas 3, 1 and 2); nor are the two types differentially distributed in depth below the cortical surface. It can be said that the adaptive pattern displayed by a cortical neuron under steady drive from the skin is a variable independent of the position of the cell in the postcentral gyrus.

The typical behavior of a slowly adapting neuron is shown by the chart and records for neuron 4-5, illustrated in Figure 10. Application of the stimulus—in this case steady pressure to the skin of the contralateral upper arm—produced a rapid onset transient response which reached about 170 impulses per second, and which declined over about 6 seconds to a more or less steady rate of discharge of 50–60 impulses per second. With release of the skin pressure the discharge rate drops at once to zero. The discharge frequency of units of this type then slowly returns to its resting or 'spontaneous' rate over a period of a few seconds—this recovery period is not shown for unit 4-5.

Our observations suggest that for any given unit the frequencies reached during the onset transient and the steady state discharge, while they vary from one neuron to another, are functions of the intensity of the steady stimulus and its position within the cutaneous receptive field for the neuron. Stimuli to the

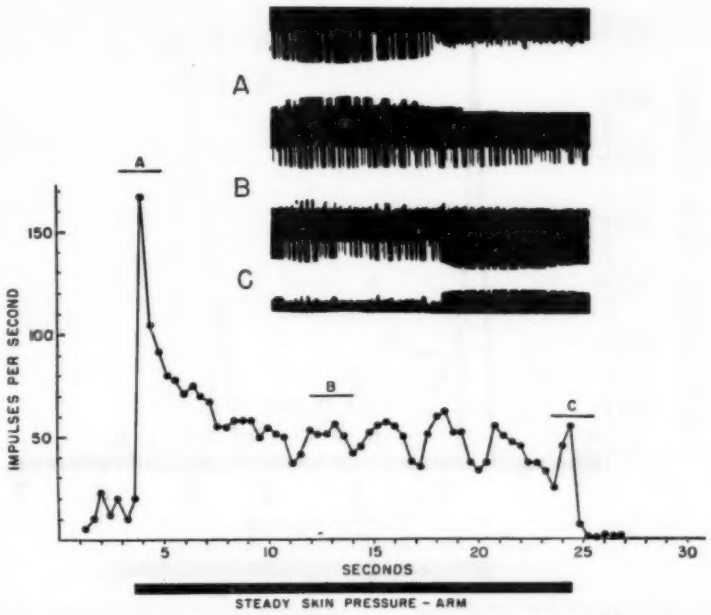

FIG. 10. Graph below plots impulse frequency of neuron 4-5 before, during and after the application of steady pressure to center of its peripheral receptive field on the contralateral arm. Application of pressure produces initial high frequency response, which is followed by a slow decline to a more or less steady rate of discharge. Insets: records of discharges for two second periods at times marked by bars A, B, and C on the graph. Short time constant recording. Cell isolated 2950 μ below the cortical surface, in area 3, during penetration M4-P1, and observed for 124 minutes.

center of the field are usually most effective, and are progressively less so as the site stimulated is moved towards the edge of the field. However, we have made no detailed quantitative study of this relation.

The majority of neurons driven from the skin, however, adapt quickly to steady stimuli. The usual pattern is shown by curve B of Figure 11. The neuron concerned responded to application of the stimulus with a high frequency onset transient, which rapidly declined to a low and oscillating rate of discharge. Rarely, such a neuron responds to removal of the stimulus with an "off transient". An example of such an event is shown by curve A of Figure 11. We interpret this latter event as being due to re-excitation of the peripheral receptors upon removal of the stimulus.

We have not observed the pattern of response of a cortical neuron to change, and many of them have been held under observation for long periods of time. In fact, when stimuli of reasonably similar force, location, and duration

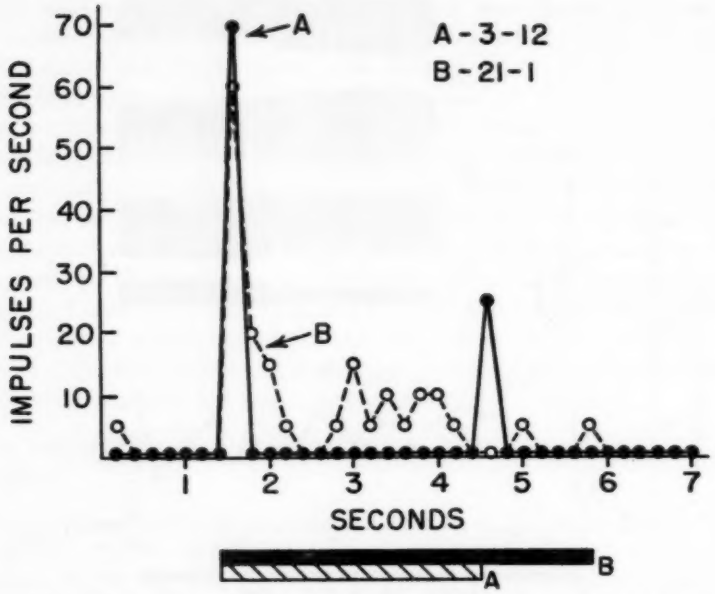

FIG. 11. Impulse frequency plots of two neurons, from different experiments, which ceased to discharge very quickly during steady stimuli delivered to their peripheral receptive fields. B—neuron 21-1 discharged a sharp onset transient, and then fired at a low rate during the continuing steady pressure to the skin of its receptive field on the contralateral forearm. Cell isolated 520 μ below the cortical surface of area 1 or area 2—the electrode track was not found in the sections; cell observed for 35 minutes. A—neuron 3-21 discharged sharp on and off transient bursts when pressure was applied and when it was removed from its receptive field of skin over the contralateral elbow. Cell isolated 770 μ below the cortical surface of area 1 or area 2; electrode track was not found in the sections; observed for 42 minutes.

are applied to the cutaneous receptive field, the pattern of response of the cortical neuron is reproduced very closely. An example of such reproduction is shown by the curves of Figure 12, which plot the response frequency of neuron 7-2 to each of two steady stimuli. The elapsed time between the two trials was 18 minutes. The record of the impulse discharge of this unit obtained during a third trial is shown in Figure 13.

In the large majority of cases the quickly adapting neurons of the cortex are driven by even the slightest bending of the hairs of the skin, while neurons displaying the steady state discharges are adequately activated by light pressure upon the surface of the skin. So long as hair bending does not displace the skin itself, these latter are insensitive to it. But this relation is not always the case, and neurons of each type have been observed whose receptive fields were located in hairless regions of the body. Nevertheless, it seems most likely that

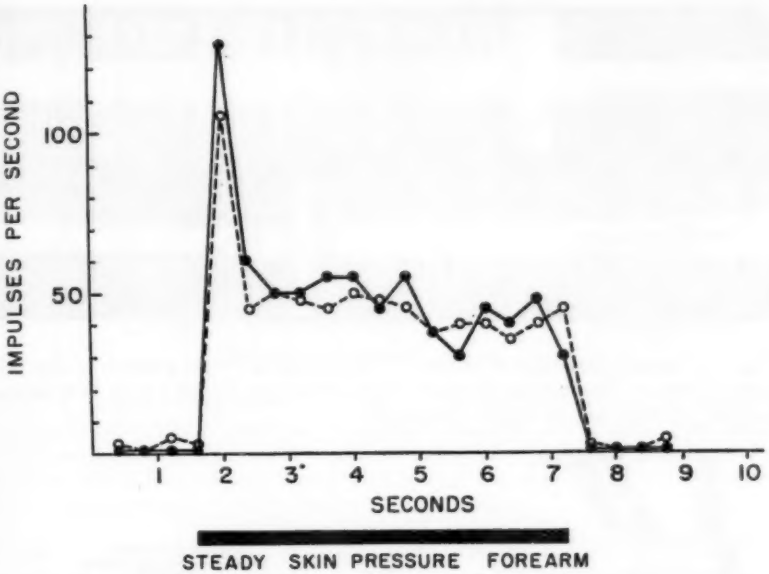

FIG. 12. Impulse frequency plots of neuron 7-2, responding to two identical steady pressure stimuli delivered to its receptive field on the volar surface of the contralateral forearm. Trials were made 18 minutes apart; the activity patterns replicate closely. Cell isolated 1050 μ below the cortical surface of area 2 during penetration M7-P3; observed for 140 minutes.

the pattern of response of a cortical neuron is determined by the adaptive properties of the peripheral mechanoreceptors to which it is linked (1).

INHIBITION OF POSTCENTRAL NEURONS BY CUTANEOUS STIMULI

The observations described above are based upon studies of 593 neurons of the postcentral gyrus which were excited by stimulation of the skin. Of this total we have observed 53 which were not only excited by stimulation of one skin area, but were also inhibited by stimulation of another. Physiological stimulation in the latter area holds in abeyance both the spontaneous and evoked discharges of such a neuron. In this section we wish to describe some aspects of this phenomenon, and the disposition of the excitatory and inhibitory skin areas related to single postcentral cells.

The phenomenon is illustrated in Figure 14. It will be seen there that the discharges of neuron 2-5 were evoked by stimulation of a restricted area of skin on the pre-axial side of the contralateral forearm. Stimulation anywhere within a much larger area of surrounding skin, extending from wrist to elbow, caused a cessation of this unit's activity. The plot of the change of impulse frequency

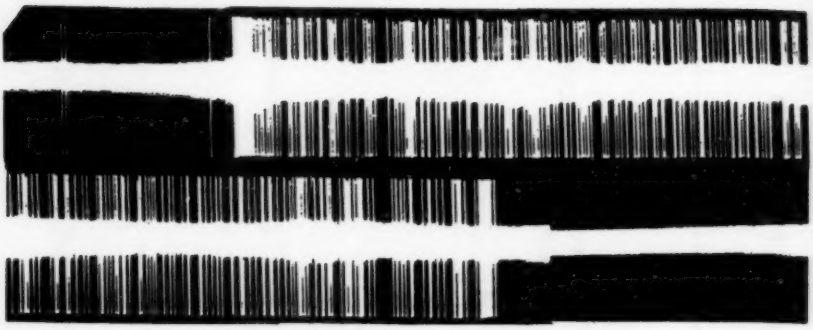

FIG. 13. Impulse discharges of neuron 7-2 responding to steady pressure to skin of its receptive field on volar surface of forearm. Spike amplitude 3–4 mv.; short time constant recording. See text, and the legend of Figure 12.

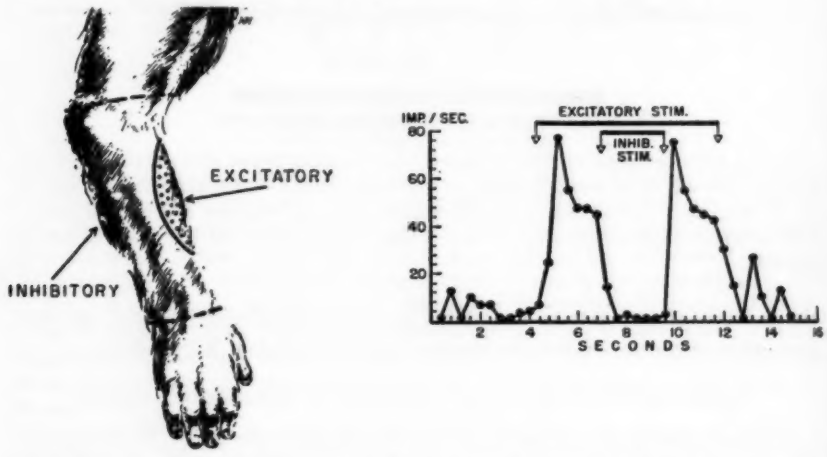

FIG. 14. An example of afferent inhibition in the somatic system. Neuron 2-5 was driven from the receptive field of the skin of the contralateral preaxial forearm, as shown in the drawing. Both its spontaneous and evoked activities were inhibited by light tactile stimulation within a much larger field surrounding the excitatory field, and extending from elbow to wrist. Only the dorsal aspects of the fields are shown, for they were similar on the volar aspect of the arm. Graph plots the impulse frequency before, during, and after a sequence of excitatory stimulus-interpolated inhibitory stimulus-continuing excitatory stimulus. Full description given in the text. Cell isolated 830 μ below the cortical surface of area 1 in penetration M2-P5; observed for 120 minutes.

with time, to the right of Figure 14, shows, firstly, the rapid onset transient and decline to a more or less steady rate of discharge with application of steady pressure to the excitatory skin area. Interpolation of steady pressure within the inhibitory field causes an abrupt and nearly complete cessation of dis-

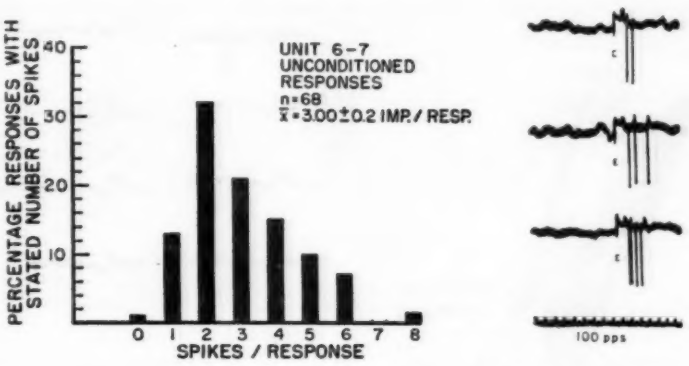

FIG. 15. Three typical responses of neuron 6-7 to electrical stimulation of the center of its cutaneous receptive field located on the postaxial side of the contralateral wrist; the field is shown in Figure 16. Bar graph to the left shows distribution of the population in terms of the number of impulses per response. Cell isolated 3120 μ below the cortical surface, in area 3, during penetration M6-P1; observed for 180 minutes.

charge, in spite of the persisting excitatory stimulus. Upon removal of the inhibitory stimulus the pattern of response to the continuing excitation repeats its initial form. This pattern of release from inhibition is very variable, however. Most commonly, we have seen a post-inhibitory rebound to a very high frequency of discharge. This rebound phenomenon—"paradoxical driving"—is frequently the first indication that inhibition is present. Occasionally, on the other hand, we have observed for other cells a slow recovery from inhibition.

Some measure of the duration of inhibition is indicated by data obtained from study of another cell, which are given in Figures 15 and 16. The first shows, to the right, three responses of this cell, each evoked by a brief electrical stimulus of the same supramaximal strength delivered to the center of its excitatory receptive field, which occupied the post-axial side of the contralateral wrist. The bar graph plots the distribution of such responses in relation to the number of impulses per response (mean = 3.00). The topographical relations of the excitatory and the surrounding inhibitory receptive fields are shown to the left in Figure 16. Representative records obtained at a series of intervals in an interaction series are shown to the right above. They were obtained using two electrical stimuli delivered to the skin on opposite sides of the wrist. The inhibitory stimulus delivered alone evoked a slow negative wave, but at no intensity or in any temporal pattern did it evoke an impulse discharge from the unit under study. On the contrary, conditioning the system with this stimulus rendered the cortical neurons almost completely unresponsive to the excitatory stimulus for a period of nearly 20 msec. The slow recovery from inhibition is shown by the plot below.

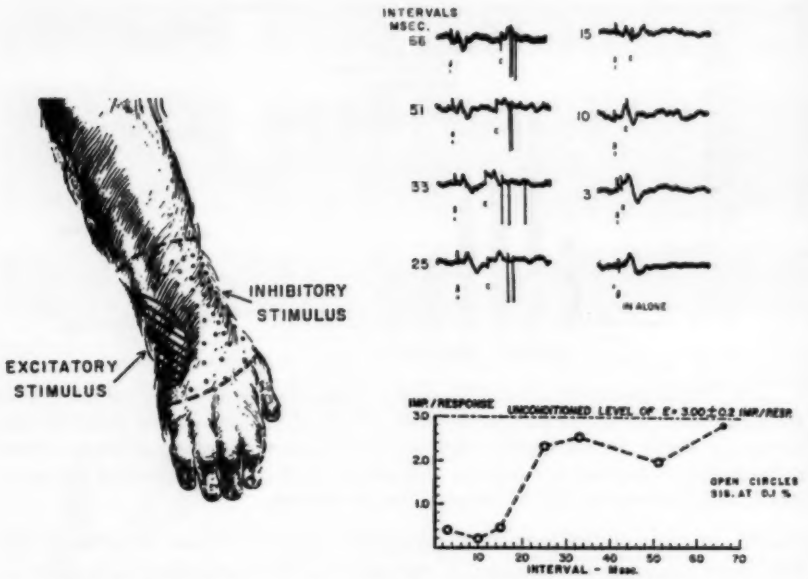

FIG. 16. Excitatory and surrounding inhibitory peripheral receptive fields for neuron 6-7 are shown in the drawing. The series of records to the right above was obtained as the response to stimulation of the excitatory field was conditioned at a series of intervals by a preceding stimulus at a site in the inhibitory field. The graph plots recovery from inhibition in terms of the mean value of impulses per response to unconditioned excitatory stimulus.

An important aspect of the relation between these two events is not shown by the graph of Figure 16. That is, that when the two stimuli are delivered simultaneously, with peripheral paths of about equal lengths, the inhibition produced is usually of a slight degree. To produce a nearly complete inhibition of the response to the excitatory stimulus the inhibitory must lead the excitatory stimulus by some 3-4 msec. Now it is clear that no separate set of peripheral afferent fibers is uniquely concerned with either inhibition or excitation of cortical cells, so that one can assume with some reason that the afferent fibers concerned are of the same size and conduction velocity ranges. It therefore follows that either (a) the relays concerned with inhibition are either more numerous than or of a different nature from the excitatory, or (b) the latency of the inhibitory event in the cortex requires a longer time to reach an effective level. Solutions to these aspects of the problem obviously require investigation using intracellular recording.

The spatial relation of the excitatory and inhibitory receptive fields playing upon cortical neurons is a matter of considerable importance. In each instance

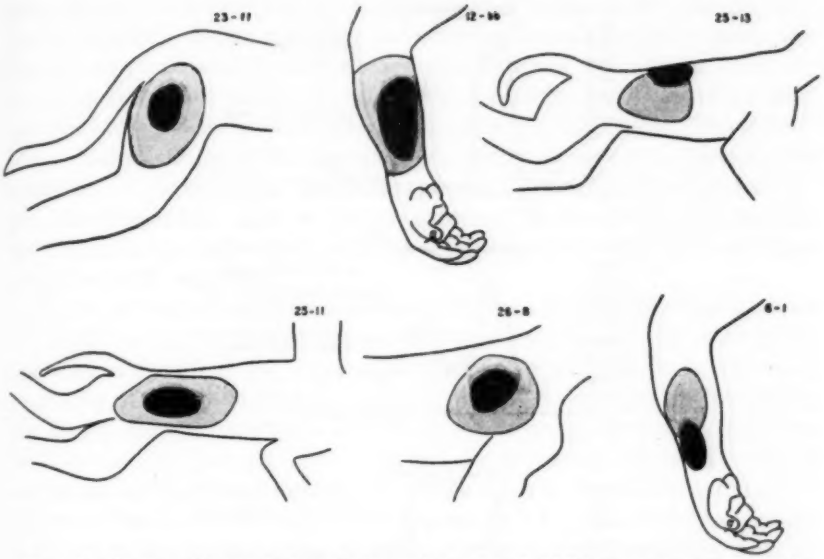

FIG. 17. Topographical relation of the excitatory and inhibitory cutaneous receptive fields for 6 neurons of the postcentral gyrus. In four cases the relation is that of an excitatory center and an inhibitory surround; in the other two the inhibitory field is placed eccentrically relative to the excitatory.

these have been mapped out using physiological stimuli. Several are shown in Figure 17. Most commonly the inhibitory field surrounds, either partially or completely, the excitatory, as shown in the figures. On the limbs the optimal site for inhibition is occasionally axially opposed across the limb from that for excitation. On the proximal limbs and trunk the pattern is clearly that of an excitatory field and an inhibitory surround, as shown for neurons 23-ff, 12-bb, 25-11, and 26-8. Some difficulty arises in marking the line between the two fields. This has been done by observing at which position of stimulation the spontaneous rate of impulse discharge changes from an increase to a decrease as the stimulus moves from the excitatory into the inhibitory field. The transition is more a gradient than a sharp line dividing the two. That the inhibitory effect does in fact arise from the skin has been shown by the control procedures described earlier (page 111), and confirmed by electrical stimulation of the skin. Afferent inhibition possesses several of the properties of afferent excitation: temporal summation of the inhibitory effect produced by repetitive stimuli which are delivered to a single site in the inhibitory field; spatial and temporal summation of the inhibitory effects of stimuli delivered to separate sites; and, for some neurons, adaptation to steady inhibitory stimuli.

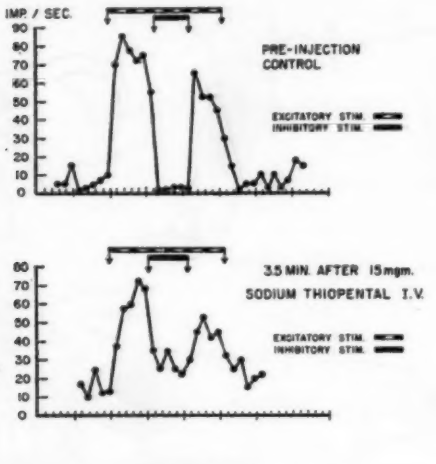

FIG. 18. An experiment upon neuron 2-5, showing the remarkable susceptibility of afferent inhibition to even slight deepening of the anesthetic state, to which excitatory driving is resistant. Graph above shows impulse frequency during excitation-interpolated inhibition-excitation sequence similar to that shown in Figure 14 for this same cell. For one to two minutes after intravenous injection of 15 mg. Sodium thiopental in this 5 kg. monkey no inhibition could be obtained. Record upon which middle graph is based was obtained 3.5 minutes after injection, as inhibition reappeared. The injection had no discernible effect upon excitatory driving. Record upon which lower graph is based was obtained 10 minutes after injection. The graph is also shown in Figure 14.

Inhibition differs very strikingly from excitation in its susceptibility to anesthesia, accounting we believe for the fact that we have observed it for only 10 per cent of the postcentral neurons we have studied. When inhibition is present it can be obliterated by additional intravenous injections of small quantities of barbiturates, which affect in no measurable way the excitatory driving of the unit under study. An instance is illustrated in Figure 18, for neuron 2-5. The effect of a steady inhibitory stimulus introduced during the response to a steady excitatory stimulus is shown by the upper chart. Immediately after the record upon which that chart is based was taken, 15 mgm. of sodium thiopental was given intravenously, to this 5 kgm. monkey. Within

seconds it was no longer possible to obtain any inhibition by the appropriately placed stimulus. Within two minutes inhibition began to appear again, and the second graph was made from a record obtained 3.5 minutes after the injection. It shows a partial recovery of the inhibitory process. After ten minutes the pattern was fully restored, as shown by the third graph. Throughout this period no change in the response of the cell to an excitatory stimulus was apparent. We have studied this transitory and differential effect of small injections of anesthetic agent on several occasions. We interpret it to mean that the central relays subserving inhibition are qualitatively different from those concerned with excitation.

One of the most consistent observations we have made is that whenever one can inhibit a cortical neuron by stimulation of the skin, one can hear in the background sounds of the impulse discharges of other cells. Such cells must be nearby, for slight vertical movement of the electrode frequently brings into the record the electrical signs of such a unit in company with those of the first, so that the two cells can be studied simultaneously. Such an event occurred during the study of neuron 2-5 (see also Fig. 14). The results obtained are shown in Figure 19. The frequency of response of 2-5 to the sequence of stimuli is shown on the graph by the dashed line, that of the second unit, 2-5a, by the continuous line. Application of the stimulus which is inhibitory for the first is excitatory for the latter; the events are almost exactly reciprocal. This indicates that cells closely adjacent in the cortex are reciprocally related to appropriately located cutaneous stimuli.

Of the 53 inhibitable neurons studied, 34 were encountered in penetrations whose tracks were later identified in the serial sections. Twenty-six of these were in area 3, and 8 in area 1. This should not be interpreted to mean that inhibition does not occur in area 2, for our total sample of cutaneously driven neurons of area 2 is far too small to settle this point. We have no evidence of a differential distribution in depth of those neurons which can be inhibited.

POSTCENTRAL NEURONS WITH UNUSUAL PROPERTIES

Of the total population of 593 neurons of the cutaneous class which were studied there are 12 which showed properties strikingly different from all the others. These properties may be summarized as follows:

(1) The neurons have extremely large peripheral receptive fields;

(2) They may be activated by stimuli delivered to the ipsilateral side of the body;

(3) When so driven by ipsilateral stimuli, they may be inhibited by stimulation of the contralateral side of the body;

(4) The inhibitory receptive field may also be very large, and correspondingly located with respect to the ipsilateral excitatory receptive field.

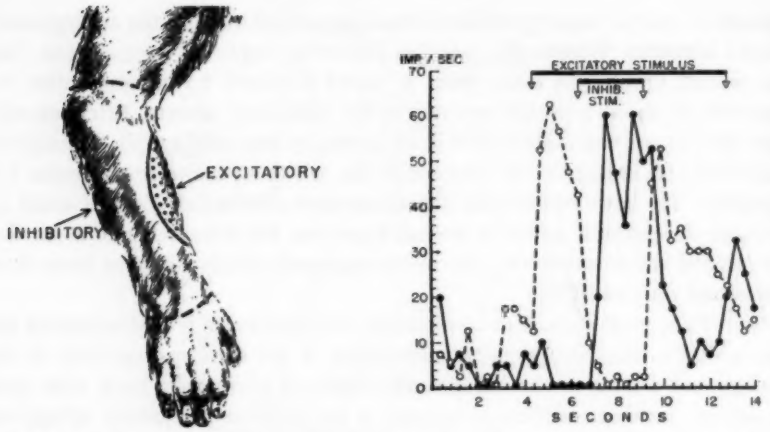

FIG. 19. Further study of neuron 2-5. It was noticed that the inhibitory stimulus drove small spikes in the background of the record. Slight downward movement of the electrode brought the electrical signs of the impulses of neuron 2-5a into the record together with the signs of those of the first cell isolated. The former displayed an activity pattern reciprocal to that of neuron 2-5. These patterns are shown by the graph of the impulse frequency of 2-5 by dashed lines, and that of 2-5a by full line.

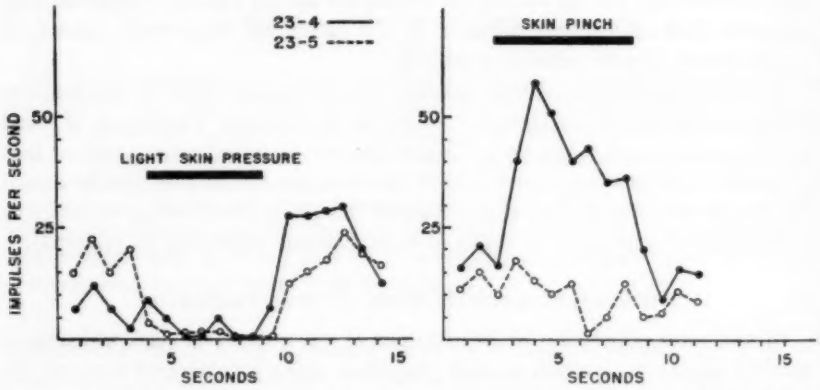

FIG. 20. Graphs plot the impulse frequencies of neurons 23-4 and 23-5, which displayed properties unusual for postcentral neurons. They were observed simultaneously at the same electrode position. Their "spontaneous" activities were inhibited (graphs to the left) by light mechanical stimuli delivered anywhere within a very large receptive field covering the entire contralateral thorax, and extending to the midline of the back. Sharp pinching with a toothed forceps, on the other hand, *drove* neuron 23-4, while the frequency of 23-5 was little affected by this stimulus (graphs to the right). The effect upon 23-4 of stimuli delivered to exactly the same peripheral site depended upon the quality of the stimulus. Cells isolated 2375 μ below the cortical surface, in area 3, during penetration M23-P3; observed for 35 minutes.

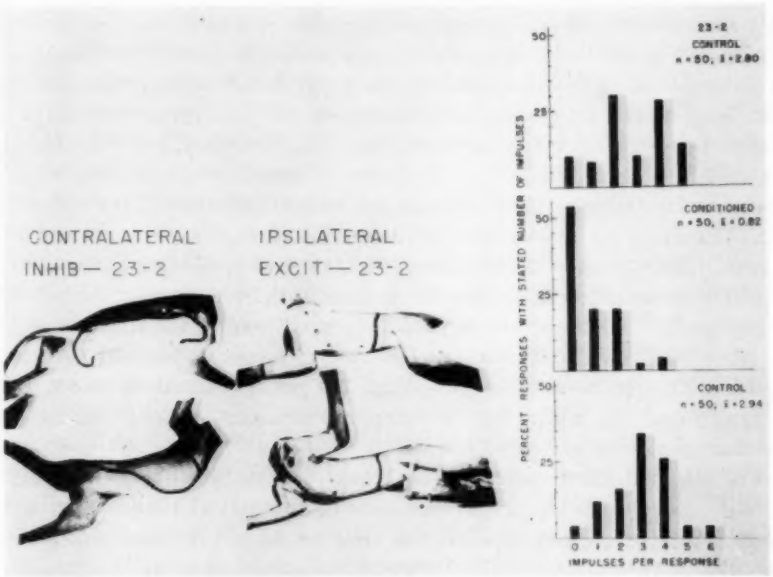

FIG. 21. Data related to neuron 23-2, a postcentral neuron with unusual properties. The photographs show the ipsilateral excitatory and the contralateral inhibitory cutaneous receptive fields for this neuron. The first and third bar graphs show distributions of responses to electrical stimuli delivered to the ipsilateral excitatory field. These populations were obtained before and after that illustrated by the middle bar graph. In this latter case an oscillating light tactile stimulus was delivered to the contralateral inhibitory field continually during observation of responses of the second population. A second unit, 23-1, was simultaneously in the record, and had an almost exactly reciprocal relation to the two receptive fields shown. Cells isolated 3625 μ below the cortical surface, in area 3, during penetration M23-P2; observed for 247 minutes.

(5) These cells may be driven by stimuli which are clearly injurious to the skin.

(6) When driven by noxious stimuli, they may be inhibited by light mechanical stimuli delivered to exactly the same receptive field.

Each of the 12 neurons did not, of course, display all of these properties. Cells of this type were encountered in both areas 3 and 1, and at a variety of depths below the cortical surfaces. They are not exclusively contained within any one cytoarchitectural field or any one layer of the cortex.

The charts of Figure 20 illustrate one form of the activity of cells of this group. The two neurons were isolated simultaneously in the record. Their spontaneous or background activity was *inhibited* (chart to the left) by very light mechanical stimuli delivered anywhere within a very large receptive field—the skin covering the entire contralateral thorax, and extending to the

midline of the back. On the other hand, sharp pinching with a toothed forceps anywhere within this same receptive field excited one of the units under study (chart to the right), while affecting the rate of the second but little. With reference to neuron 23-4, and others of this type, then, it appears that the effect produced upon the cortical cell—excitation or inhibition—may vary for stimuli delivered to exactly the same site in the periphery, and depends upon the quality of the stimuli.

Figure 21 illustrates the receptive fields of two neurons which were studied simultaneously, for more than four hours. The first, 23-1, was activated by contralateral stimuli, and the second, 23-2, by ipsilateral stimuli. In addition, it was observed that light mechanical stimuli delivered to its contralateral receptive field, while driving neuron 23-1, would inhibit the response of 23-2 to stimuli delivered to its ipsilateral excitatory receptive field. The extent of that inhibition is indicated by the second bar graph of the three shown to the right of Figure 23, which is to be compared with the control populations indicated by the first and third graphs.

The observations made upon this group of cells, in spite of their limited number, carry important implications for conceptions of the function of the somatic afferent system, especially so when considered in relation to findings obtained in a recent study of the posterior nuclear group of the thalamus (18).

DISCUSSION

The results obtained in the present study allow a reconstruction of the neural events evoked in the postcentral gyrus by light tactile stimuli delivered to the skin. Such stimuli evoke short trains of impulses in each first order afferent fiber activated. These in turn provoke discharge in a group of cells of the dorsal column nuclei, and these activate a group of cells of the ventral posterior nuclear complex of the thalamus. The mechanisms for limiting the the number of cells activated in the cerebral cortex, which we wish to discuss, occur also at these intervening nuclear relays of the system. Thus the thalamic cells which discharge impulses are limited to a restricted zone, limited first of all by anatomical connections, and secondly by physiological mechanisms. Cells of the cortex which are activated by the thalamocortical impulses may be regarded as forming a discharge zone. The cells of this zone do not all respond in an identical manner. Those at or near the center of the group of cells discharge short repetitive trains of impulses, trains which vary in number from 2 to 8 impulses, for in our 38 intensity series we observed not a single cell whose mean value of impulses per response was not more than one. The response of these cells of the center of the discharge zone occurs with great security, and at the shortest latency. Those cells just off the center discharge fewer impulses at longer latencies, with less certain probabilities. The response parameters con-

tinue to change gradually from cell to cell between the center and the edge of the discharge zone; those at the edge respond insecurely, while those just outside must receive only sub-threshold synaptic excitations. This description follows from the reciprocal interpretation of the results of our studies of the gradient of the projection of a cutaneous receptive field upon a single cortical cell. When the stimulus is shifted from the center towards the edge of that cutaneous field, the cell under study occupies first the center and then a series of positions within, and finally the edge, of the discharge zone of cortical cells set up by the stimulus.

This pattern is closely related to the strength of the peripheral stimulus, that is, to the numbers of first order afferent fibers activated, and the durations and frequencies of the trains of impulses set up in them. With stimuli of decreasing strength the discharge zone shrinks, and cells of its center respond at longer latencies, with fewer impulses, and with less certain probabilities. *Which* cells of the cortex are activated is determined by the *place* in the periphery stimulated. The intensity of the stimulus is depicted by the extent of the cortical discharge zone, and the spatial and temporal patterns of activity of the cells within it.

Such a description seems reasonable for single brief stimuli. The physiological stimuli of ordinary life are scarcely ever so brief, however, for they involve stimulus patterns of longer duration and greater complexity. Thus the capacity of the system to transmit patterns of impulses of such complexity and timing could be a limiting factor in sensory discrimination. It is important to know how quickly the system recovers from one action and is ready for another, how faithfully it performs under repetitive drive. Our studies of the responses of single cortical cells to two stimuli delivered at a variety of intervals indicate that, in the most lightly anesthetized animals, no true unresponsive time of the system exists. In fact, activation of the system leaves it more than normally responsive to a succeeding stimulus —this is the period of early facilitation. Studies of cells driven by trains of stimuli of different frequencies (delivered to the centers of peripheral receptive fields) indicate that cortical cells can respond at frequencies well in excess of those normally set up in first order afferent fibers by natural stimuli. This is an indication of the great security of action in the dorsal column-medial lemniscal system. It seems very likely that all but the most insignificant stimuli suffice to activate cortical cells. It is possible that this capacity for transmission, in the waking animal, reaches the ultimate limit: a single impulse in a single afferent fiber is likely to produce responses of cortical cells. The implication is that the threshold for cortical response, which is assumed to lead to perception of the peripheral event, *is set by the threshold of the sensory receptors in the periphery.*

Earlier studies have shown very clearly that when stimuli are delivered to

a single peripheral site, a very large area of cortex is activated, as measured by the slow wave evoked response recorded on the pia-arachnoid surface with a gross electrode (27). And such a divergence appears inevitable from the anatomical relations which exist. Obviously, no simple idea of a specific relation of periphery and brain suffices to explain the ability of such organs as the hand and fingers to discriminate so well. What dynamic or functional properties of the system can account for its exquisite power for form and contour recognition?

In the first place, the spread of the cortical discharge zone is limited by the insecurity of the synaptic linkage between the stimulated peripheral site and the cells of the edge of the discharge zone. Renkin has shown (20), and we have confirmed her observations, that such fringe cells fail to follow trains of all but low frequencies. Equating frequency of first order afferent input with intensity of stimulation, it then follows that the spread of the cortical discharge zone is limited, or even decreased, by the failure of fringe cells to follow. This insecurity of synaptic impingement is shown also by the lengthening recovery cycles of cortical neurons which are obtained when the site of stimulation is shifted to the edges of their peripheral receptive fields.

Nevertheless, this limiting factor is not powerful. A second and much more impressive one is provided by the mechanism of afferent inhibition. This shows that the excitatory peripheral receptive field for a cortical neuron is surrounded by a belt of skin within which stimulation will inhibit the cell under study. Inhibition has not been seen under deep anesthesia, appears with increasing incidence as the anesthetic state lightens, and thus is likely, we believe, to be an attribute of all postcentral neurons sensitive to cutaneous stimuli. Making the reciprocal interpretation once more, one can conclude that the group of cells excited by stimulation at a single peripheral site is surrounded by a ring of cells which is inhibited by the same stimulus. This phenomenon has been observed at subcortical relays of the system as well. One conception of the role it may play in such functions as two point discrimination is indicated in Figure 22. The fact that no first order afferent fibers are concerned with inhibition alone, and that inhibition has been observed at each successively higher level of the dorsal column—medial lemniscal system, suggests at once that some collateral mechanism is involved at each synaptic station. This is at least compatible with the observation that the latency of inhibition is slightly longer than that of excitation. From a consideration of data obtained in studies of spinal motoneurons (4, 5) one can surmise that this inhibition is mediated by intercalated neurons. We have no evidence upon which to confirm or deny this supposition.

All the above pertains only to the lemniscal component of the somatic afferent system, a component made up of the first order afferents of the dorsal

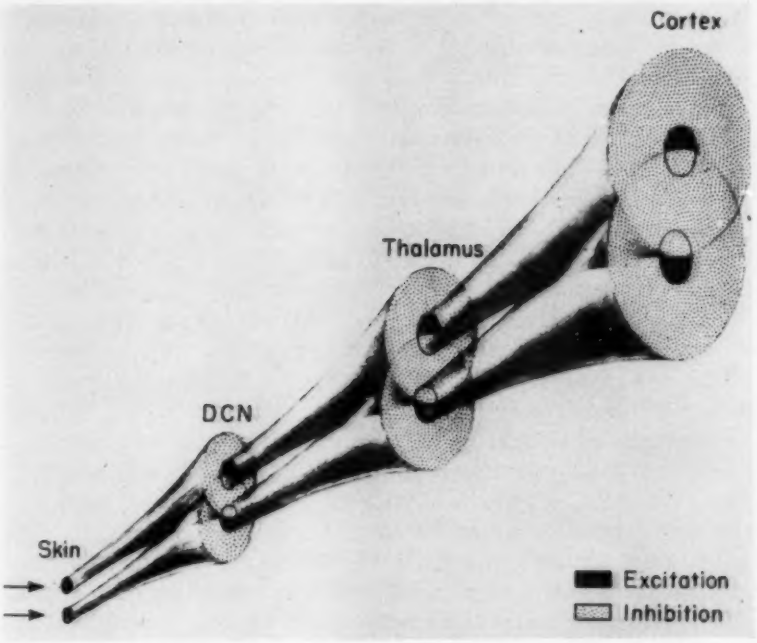

FIG. 22. A schematic drawing illustrating the role inhibition is thought to play in discrimination in the somatic afferent system. Each stimulus delivered to the skin activates a group of cells of the dorsal column nuclei, and inhibits those which surround that excited group. This pattern is repeated at each successive level of the system. When two stimuli are delivered and brought closer together the surround inhibition will delay fusion of the excited zones set up by the two stimuli, thus preserving the two peaks of activity at the cortical level, an event thought to be essential to the perception that two stimuli have been delivered. More complicated patterns of central excitation and inhibition will accompany more complicated peripheral stimuli, but the same organization should contribute to pattern and contour recognition.

columns, lemniscal fibers of dorsal column nuclear origin, the cells of the ventral posterior nuclear complex of the thalamus which project upon the cortex, and the cells of the postcentral gyrus receiving that projection. It is a system of great synaptic security, poised for action at high frequency levels, possessing all those neural attributes required for the discriminatory functions preëminently served by the somatic afferent system. That the spinothalamic component possesses a true thalamic projection which increases in proportion in the course of phylogeny is well known. Evidence has recently become available that it projects upon a portion of the posterior thalamic group (26). It may of course project upon the ventral posterior complex as well. At least

preliminary evidence allows some description of its physiological properties: they are in each regard strikingly different from those of the neural elements of the lemniscal component of the system. These cells have huge receptive fields, which in the limit may cover the entire body surface. Complex side-to-side, inhibitory-excitatory interactions occur. Some cells are clearly activated by stimuli which can only be regarded as noxious in nature, and such cells may be quite differently affected, depending upon the quality of the stimulus, i.e., *they may not be modality specific*. Such observations have been made upon interneurons of the dorsal horn by C. C. Hunt (8), upon cells of the posterior thalamic nuclear group by Poggio and Mountcastle (18), upon those of the second somatic cortical area by Carreras (2), and in the present study upon a few cells of the postcentral gyrus. These last two observations suggest that the posterior nuclear group projects upon both the first and the second somatic areas of the cortex, which fits well with its sustaining nature, as proposed by Rose and Woolsey (22). This spinothalamic component of the system appears to be arranged to provide information concerning the qualitative nature of the peripheral stimulus, but not its quantitative parameters. Since the attributes of both systems provide sensory data essential for the complete perception of of a peripheral stimulus, an important problem is posed: at what level and through what mechanisms are these different but complementary aspects of somatic sensation integrated into a meaningful percept of the peripheral event?

SUMMARY

Results are presented of studies of neurons of the postcentral gyrus of the macaque monkey which are activated by stimulation of the skin. The sizes, shapes, positions and stability of the cutaneous receptive fields which play upon such cortical neurons are described, as well as the gradients of synaptic impingement upon the cell set up by stimulation at different positions within its receptive field. The temporal capacity of the system to transmit to the cortex afferent activity evoked repetitively is described. It is thought to provide a comfortable safety factor: its temporal capacities exceed the usual frequencies set up in first order afferents by physiological stimuli. All these observations are brought together in a synthesis of the role of the system and these cortical neurons in sensory discrimination, with particular emphasis upon the role of surround inhibition in increasing the capacity of the system for such discrimination. Finally, observations upon a small number of neurons of unusual properties are described. These cells displayed all those properties characteristic of cells of the posterior thalamic group. This is interpreted to indicate the projection of both lemniscal and spinothalamic components of the system upon the postcentral gyrus.

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EFFECT OF A SHELL-FISH POISON ON END-PLATE POTENTIALS

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It has been established that the substance responsible for shell-fish poisoning (1–5) is a low molecular weight compound of known chemical composition (6). An extensive literature exists describing the epidemiological, clinical and chemical aspects of this substance (1–7). Little, however, is known concerning the mechanism by which this substance produces its toxic effects. The purpose of this paper is to describe experiments performed to establish a method of observation by which the mechanism of action of shell-fish poison might be studied. The following data indicate that, by using paired iliofibularis muscles from the same frog, under the conditions described, similar rates of decrease in end-plate potential amplitudes can be obtained. Pertinent ancillary observations are reported.

MATERIAL AND METHODS

Freshly decapitated *R. pipiens* were used. The iliofibularis muscle in each leg was carefully dissected out with the attached nerve. The paired muscles were immersed under oil in oxygenated Ringer's solution containing curare (0.04 mg. per milliliter). After two to three hours incubation at room temperature, each muscle was transferred to a plastic vessel containing an aliquot of the curare-Ringer's solution and firmly attached at each end between forceps mounted on a movable bar. Platinum electrodes were applied to the muscle as previously described (8). The exploratory electrode was adjusted until a consistent end-plate potential (EPP) was observed on the oscillographic screen. A stimulus was applied to the nerve each second and the EPP tracings obtained were photographed for measurements. The muscle was raised into the supernatant oil layer and shell-fish poison[1] was added to the curare-Ringer's solution. The concentration was 0.1 microgram per milliliter. The time of reimmersion of the muscle into this solution was noted. At the intervals indicated in the figures the muscle was raised from solution into oil and EPP tracings were recorded. In preliminary studies it had been established that the EPP remained constant during this recording period. The rate of fall in EPP amplitude was compared with that obtained when the second of a pair of muscles was treated in the same manner. Ten experiments have been analyzed and the results are reported.

For histochemical studies iliofibularis muscles were subjected to one of the three following treatments. 1. Muscles were immersed for three hours in a frog-Ringer's solution containing curare. 2. Half of these muscles subsequently were immersed in other aliquots of

[1] The material used was obtained through the kindness of Dr. Edward A. Metcalf of the Industrial Liaison Office of the Directorate of Research, C.W.L. The toxicity was equivalent to 5,500 ± 500 mouse units per milligram.

TABLE I

Types of Fixatives and Methods of Staining Employed on Neuromuscular Preparations Previously Exposed to Shell-Fish Poison

Fixative	Method	Test for:	Results
Alcohol-formol-picric acid	McManus' periodic acid-Schiff	glycogen and other polysaccharide complexes	Negative*
Alcohol-formol-acetic acid	Millon reaction	phenols, tyrosine	Negative
Neutral 10% buffered formalin	Feulgen reaction	deoxyribonucleic acid (DNA)	Negative
Neutral 10% buffered formalin	Unna-Pappenheim	DNA and RNA	Negative
Ethanol, 80%	Weiss and Seligman	protein-bound primary amino groups	Negative
Trichloroacetic acid-alcohol	Barrnett and Seligman	sulfhydryl and disulfide groups of protein	Negative
Acetone	Gomori's Ca phosphate	alkaline phosphatase	Negative
Acetone	Gomori's azo dye method	alkaline phosphatase	Negative
Acetone	Gomori's Pb glycerophosphate	acid phosphatase	Negative
Acetone	Gomori's ATP	acid phosphatase	Negative
Acetone	Gomori's muscle adenylic acid	5-nucleotidase	Negative
None	Koelle's Acetylthiocholine	acetylcholinesterase	Negative

* The results were negative i.e. no change noted in comparison with controls.

the same solution containing, in addition, shell-fish poison at a concentration of 0.1 microgram per milliliter for approximately one hour. 3. Muscles which were immersed in a frog-Ringer's solution without added curare or shell-fish poison served as controls. Before fixation, each muscle was stretched gently across a glass ring. Both muscle and fixative were chilled before the fixation. The fixatives used are listed in Table I. The fixation was accomplished at 5° C. for not less than sixteen hours (overnight) or more than twenty-four hours. Following this, the tissues were dehydrated and imbedded in paraffin. Sections were cut at approximately six microns. A summary of the tests performed is given in Table I. Unfixed tissues were used to determine cholinesterase activity. The muscle fibers were teased apart while still immersed in the test or control solutions and incubated subsequently according to the Koelle thiocholine technique (9).

RESULTS

As shown by Run A in Figure 1 there is progressive diminution in the amplitude of the EPP when a nerve-muscle preparation is exposed to the shell-fish poison in a concentration of 0.1 microgram per milliliter. The similarity of the time required for such a loss of EPP when the second muscle of the pair is employed is indicated by Run B. Samples of the EPP tracings made at comparable times with each muscle are presented in Figure 2.

The results in six sample experiments using paired muscles have been graphed in Figure 3. The arrows indicate the time of exposure when the EPP tracing

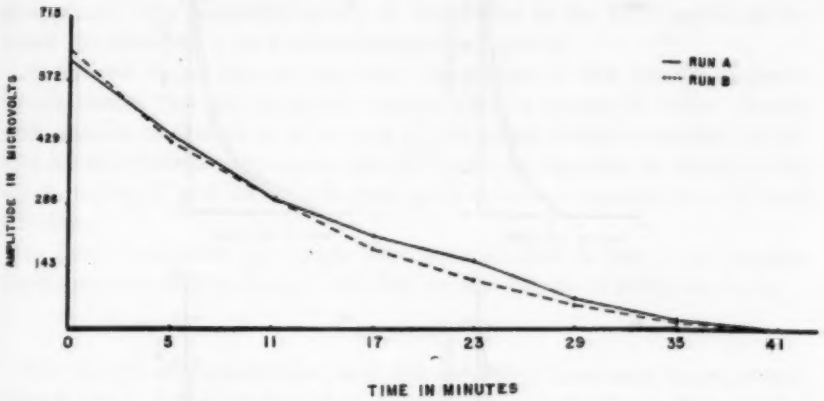

FIG. 1. The amplitudes of the end-plate potentials (EPP) recorded at the times indicated following exposure to shell-fish poison have been graphed. Run A and Run B represent the results using each member of paired muscles. Each point represents the average height of circum 60 EPP tracings made over a period of a minute. The S.D. ranged from ± 25.9 μv to ± 2.1 μv.

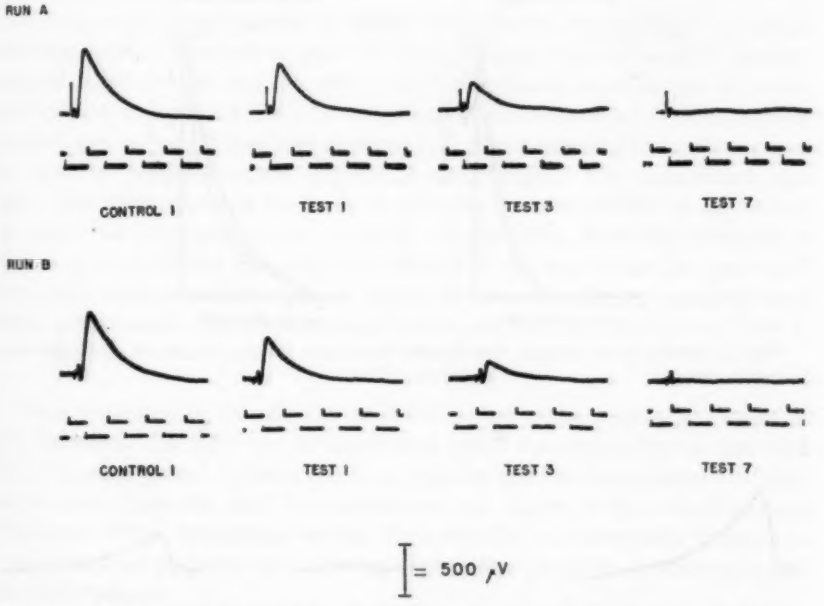

FIG. 2. Sample EPP tracings from which Figure 1 was graphed are shown. Test 1 was made after 5 minutes exposure to shell-fish poison. Tests 3 and 7 were made after 17 and 41 minute exposures respectively. Time markings are 200 per second.

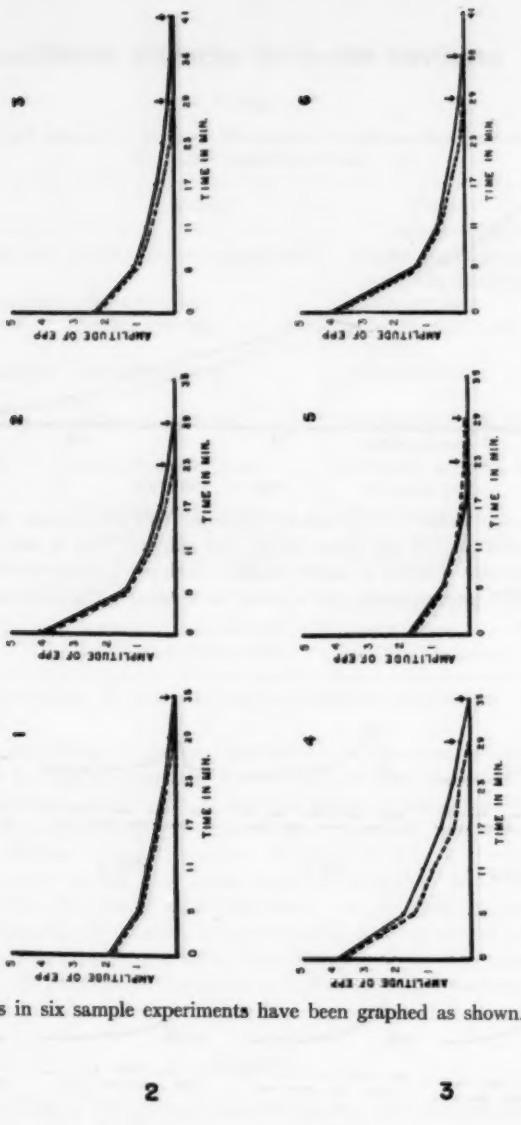

FIG. 3. Results in six sample experiments have been graphed as shown. Ordinates are in centimeters.

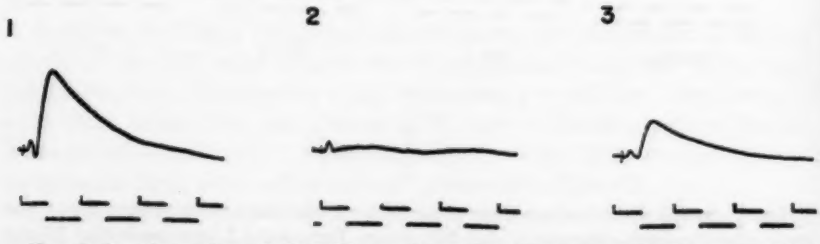

FIG. 4. Sample tracings of the EPP are shown. Tracing 1 was made during an initial control period. Tracing 2 was made after prolonged exposure to shell-fish poison. Tracing 3 was made after the same muscle subsequently had been immersed overnight at icebox temperature in curare-Ringer's solution containing no shell-fish poison. Time markings are 200 per second.

disappeared. The similarity in rate of diminution of the EPP amplitude between the members of each pair of muscles is apparent.

At the end of five experiments direct stimulation of such muscles elicited a muscle contraction only when the voltage reached circum 60. After allowing such muscles to remain in an aliquot of the curare-Ringer's solution for sixteen hours at icebox temperature the EPP partially returned as shown in Figure 4, tracing 3 and the muscle contracted on direct stimulation with only 0.6 volts.

In Table I are listed the various histochemical methods used. In no preparations were any alterations detected that were ascribable to shell-fish poison.

DISCUSSION

The clinical characteristics of shell-fish poisoning have been described previously (2, 7). Although the poison has been characterized as a "neurotoxin" (10) its paralytic effect seems to reflect a much wider spectrum of action on neuromuscular function than such a classification suggests (11). Although curare and botulinus toxin do not prevent muscle contraction on direct stimulation, shell-fish poison will modify such a reaction. This effect is at least partially reversible.

The absence of any marked inhibition of cholinesterase activity by concentrations causing paralysis support the interpretation that the mode of production of paralysis does not depend on the accumulation of an excess of acetylcholine. At no time did the EPP amplitude appear enhanced by the presence of shell-fish poison. It has been reported (11) that a muscle previously exposed to shell-fish poison does not respond to acetylcholine. This observation suggests that some aspect of the mode of paralysis is not ascribable to any failure of either the formation or the release of acetylcholine. However, attempts to detect cytological and histochemical changes at the neuromuscular junctional area that might elucidate such an aspect of the mechanism of paralysis have been unsuccessful. The histochemical techniques used are outlined in Table I.

CONCLUSIONS

Data pertinent to the effect of shell-fish poison on neuromuscular function are recorded. The EPP can be eliminated under the circumstances described and by using paired muscles the time required for its disappearance in each of the two is similar. Shell-fish poison does not appear to be a cholinesterase inhibitor. Other cytological studies have revealed no detectable changes in neuromuscular preparations following exposure to paralytic concentrations of shell-fish poison.

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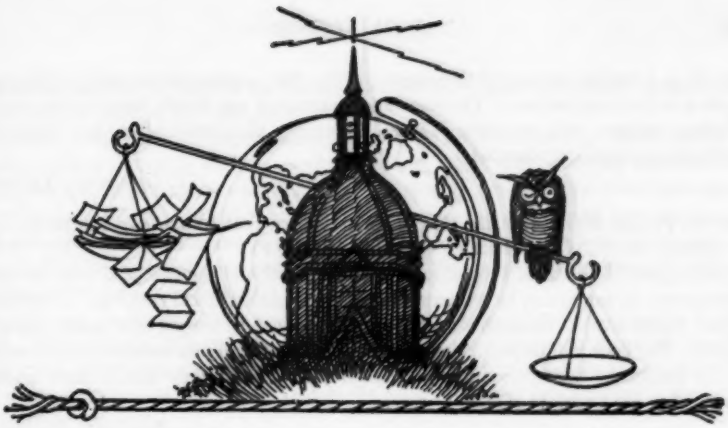

BOOK REVIEWS

History and Trends of Professional Nursing, 4th edition. By DEBORAH MACLURG JENSEN. 610 pp., $5.25. The C. V. Mosby Co., St. Louis, Mo.

This is the most recent edition of a well-known nursing text which has been used, in some of its forms, by students in schools of nursing for the past sixteen years. The author has undertaken a large order in attempting to present, as concisely as possible, the essential facts of historical and current problems; as a result, with a few exceptions, any depth of treatment has been sacrificed. Considering the complexity of the nursing situation today in both education and service, this reviewer feels that the material can be viewed only as very superficial.

In making an almost page by page comparison with the third edition it is found that probably not more than one-fifth of the fourth edition represents anything in the way of new material. There has been a slight rearrangement in sequence of chapters, an occasional subheading has been changed, and a few diagrams and other drawings have been added. The section on legal aspects has received some revision and the material is good. It is, however, questionable in the mind of this reviewer whether a unit on legal aspects even belongs in a book on history and trends of nursing.

The most extensive additions are within the section on nursing in other countries. This comparative study is interesting and not readily available elsewhere.

The book is divided into twelve units. The first unit gives the social and professional setting of nursing; the next six present nursing history up to and including World War II; one unit each is devoted to postwar developments, opportunities for the graduate nurse, legal aspects, international relationships, and nursing in other countries. The topics and subheadings are well organized. A bibliography of important studies, papers, and books for more intensive reading appears at the end of each unit. Included, also, in each unit is a list of suggested projects and questions for additional study. As a concise presentation of facts this text should prove useful to basic nursing students by providing a framework for further reading and study.

MARGARET E. COURTNEY

Clinical Dermatology. For Students and Practitioners. By HARRY M. ROBINSON, JR. AND RAYMOND C. V. ROBINSON. 242 pp., $8.50. The Williams & Wilkins Co., Baltimore, Md.

This 228 page text is the most recent in its field for students and practitioners. *Clinical Dermatology* is authored and edited by the Dermatology Staff of The University of Mary-

land. It is a concise survey of dermatology. The first portion is devoted to introductory chapters to the basic sciences. The remainder enumerate and briefly describe skin disorders including relations with systemic diseases. Numerous illustrative black and white photographs are appropriately located.

T. LAWRENCE FLEISHER

That the Patient May Know. By HARRY F. DOWLING, TOM JONES, AND VIRGINIA SAMTER. 139 pp., $7.50. W. B. Saunders Co., Philadelphia, Pa.

The purpose of the book is to provide an anatomical and physiological atlas for the use of physicians in explaining to patients the nature of their clinical problems. There are numerous diagrammatic illustrations which are explained in simple and easily understood language. The book will be particularly useful to busy general practitioners who do not have time to explain to their patients in a detailed fashion what they should know about the diseases they are trying to control or cure.

BENJAMIN M. BAKER

Pathologic Physiology of Oral Disease. By RICHARD W. TIECKE, ORION H. STUTEVILLE AND JOSEPH C. CALANDRA. 480 pp., $11.50. The C. V. Mosby Co., St. Louis, Mo.

This new text on oral pathology is intended primarily for practising dentists and dental students. Each pathologic entity is considered precisely in regards to etiology, clinical signs and symptoms, microscopic appearance, and treatment. In their discussions the authors have presented only the most widely accepted theories; their choice has not been to evoke debate or to discuss the development of pathologic concepts.

Disease entities of similar etiology are discussed in the same chapter. Each disease is covered mainly from the viewpoint of gross clinical pathology, though there has been some correlation of the clinical picture to microscopic findings. Much more could be said about the biochemistry of disease, but it is questionable whether such material would have a place in a book of this scope.

A welcome addition to the text is a chapter describing normal structures which may be mistaken for oral disease processes. There is also a discussion of non-oral tumors which may be metastatic to the oral regions and a careful description of all biopsy and laboratory tests useful to the dentist. The authors have taken particular pains to discuss precautions which should be taken when dental procedures are necessary in patients with systemic disorders such as diabetes mellitus, hyperthyroidism, and, of course, the blood dyscrasias.

The authors state that they have limited their discussion of lesions of the teeth themselves because this material is adequately covered in other texts. While this is undoubtedly true of most periodontal conditions, developmental defects and dental caries, it would seem that the relationship of various systemic diseases to lesions of the teeth would have a place in this book. For example, a discussion of the association between kernicterus, erythroblastosis fetalis and hypoplastic teeth with pigmentation could be included.

The material in each chapter has been summarized in convenient charts which could aid the clinician in differential diagnosis. Black and white illustrations of good quality have been provided for most conditions. I believe that this book has a definite place in the average dental office.

E. HENRY HINRICHS, JR.

Group Processes. Transactions of the Fourth Conference Oct. 1957. Edited by BERTRAM SCHAFFNER. 266 pp., $4.50. The Josiah Macy, Jr. Foundation Publications, New York, N. Y.

This book is another publication of transactions of a multiprofessional conference sponsored by the Josiah Macy Foundation. It consists of five parts, the first being devoted to

brief autobiographical sketches of the participants who numbered 26 and were chiefly representatives of the medical and behavioral sciences.

Fritz Redl of the Child Research Branch, National Institute of Mental Health starts the discussion with a discourse on how game ingredients affect children's play behavior. He analyzes one specific game as to potential gratification needs, frustration and the ego-support it provides for an individual child; the latter is dependent on a balance between impulse gratification and emergent anxieties on one hand and behavioral demands of the game on the other. Ego-support is also derived through role definition, group code demands and the group machinery for settling disputes.

The discussant's presentation, though more complex than necessary in parts, is clear enough. Unfortunately, this cannot be said about all the other discussants who all-too-often become involved in issues not related to the topic. Yet this very factor helps the reader understand group processes as they occur among the discussants and this represents one of the great assets of the book.

Fritz Redl also leads the group in a discourse on how our current models of personality may be altered as a result of better understanding of group processes. Contagion, "role-suction," changing of super-ego in groups and special stimulating factors which make a member act out potential wishes are considered to be of great influence in personality formation.

Page 4 of the book is a report and discussion by Alex Bavelas, a researcher for the Bell Telephone Company laboratories when he performed his experiment. It deals with problems of group size, interaction, and structural environment, prompted by communications difficulties as they frequently occur in management.

The final section is a very stimulating report with discussion by Konrad Z. Lorenz of the Max-Planck-Institut für Verhaltensphysiologie, Seewiesen, Germany. He describes the behavior of geese and what role aggression plays in their group formation. He tells of the apparent factors in flocking and fighting and the importance of the so-called triumph ceremony in their aggressive behavior. Only sexual excitement may cause a goose to abandon the triumph ceremony. Lorenz' presentation by itself makes this book worthwhile reading for anyone interested in the psychodynamics of groups.

EDUARD ASCHER

Diseases of the Colon and Anorectum: Vols. I and II. Edited by ROBERT TURRELL. 1238 pp., plus 68 pp. of index. $35.00 per set. W. B. Saunders Co., Philadelphia, Pa.

"Diseases of the Colon and Anorectum" is an extensive two-volume study of the areas mentioned in the title. Eighty-two distinguished individuals contributed to the volumes. Every conceivable surgical aspect of the colon and anorectum is considered. Basic science problems are discussed initially, including among those embryology, anatomy and physiology.

The major portion of the work is devoted to detailed consideration of the clinical surgical aspects of the problem.

In general, sound advice is accorded in each group. There are, however, exceptions to this statement as evidenced by the chapter on sphincter saving procedures. Admittedly a controversial subject, these procedures are advised for carcinomata of the rectum in the 5 cms. to 10 cms. level from the anus.

Another mode of management which is certainly controversial is the so-called non-operative method of treating hemorrhoids. In the text a chapter is devoted to the use of sclerosing agents for internal hemorrhoids. Much disagreement could be developed on this topic in many circles.

However, as mentioned above, these volumes are generally soundly written and offer

accurate and conservative advice. The illustrations are good, and the bibliography is adequate but not extensive.

The reviewer would be inclined to recommend these volumes as a reference text for those surgeons interested in these fields, resident staff concerned with these problems, and as a reference for other clinicians and students of medicine.

WILLIAM F. RIENHOFF III

The Biology of Hair Growth. Edited by WILLIAM MONTAGNA AND RICHARD A. ELLIS. 520 pp., $15.00. Academic Press, New York, N. Y.

This is a compilation of the Proceedings of the International Conference on the Biology of the Hair Follicle and the Growth of Hair held in London under the auspices of the British Society for Research and Aging. The book consists of twenty-one sections written by the outstanding authorities of the world. Basic problems of development, growth and differentiation are presented and processes of aging are discussed. All scientists interested in the phenomena of growth and differentiation will find a wealth of new material in this volume.

MAURICE SULLIVAN

Viral and Rickettsial Infections of Man, 3rd edition. Edited by THOMAS M. RIVERS AND FRANK L. HORSFALL, JR. 967 pp., $8.50. J. B. Lippincott Co., Philadelphia, Pa.

With the recent discovery of new techniques the extraordinary acceleration in virus research in the past several years has brought a concomitant need for revision of the second edition (1952) of one of the standard textbooks in this field. For the most part, the third edition represents not only a revision and a re-writing but also an expansion (from 719 to 967 pages). Chapters on new groups of viruses, the Echo and Adenovirus families, have been added. The arthropod-borne viruses have been re-classified according to the now generally accepted classification of Casals; and the chapters on these viruses have been enlarged greatly to include the great amount of information, particularly on immunology and ecology, that has been recently accumulated. The recent remarkable advances in tissue and cell culture techniques are reviewed. In the new chapter on "Biochemistry of the Virus-infected Cell" as well as in the revised section on properties of virus materials, greater attention is given to biochemical and biophysical aspects of viruses and particularly of the infections they produce. From a small discussion of hemagglutination in the second edition there has grown an enlarged chapter on virus-host cell relation. The discussion of poliomyelitis has been greatly expanded; and a chapter on hemorrhagic fever has been added to the book. The section on epidemiology, however, remains generally unchanged as a fairly straightforward introduction to the subject, except for the addition of a brief treatment of experimental epidemiology. Fairly complete chapter bibliographies serve as helpful guides to pertinent literature.

In the preface to the first edition (1948) Dr. Rivers stated "There is need for a book on viral and rickettsial infections of man to which medical students and practicing physicians may turn for information." The second edition, an enlarged revision somewhat more technically oriented than the first, tried to maintain this purpose by the inclusion of a chapter on diagnosis of viral and rickettsial infection. The third edition no longer meets this need but is now rather a work to which graduate students and practicing virologists may turn for information. Medical students and practicing physicians may find this text difficult to read.

SIDNEY E. GROSSBERG

BOOKS RECEIVED FOR REVIEW

Cancer in Families. A Study of the Relatives of 200 Breast Cancer Probands. By DOUGLAS P. MURPHY AND HELEN ABBEY. 76 pp., $2.50. Published for the Commonwealth Fund by the Harvard University Press, Cambridge, Mass..

Carcinogenesis by Ultraviolet Light. By HAROLD F. BLUM. 340 pp., $6.50. Princeton University Press, Princeton, N. J.

J.-M. Charcot, 1825–1893. His Life–His Work. By GEORGES GUILLAIN. Edited and Translated by Pearce Bailey. 202 pp., $7.00. Paul B. Hoeber, Medical Book Dept. of Harper & Bros., New York, N. Y.

Ciba Foundation Symposium. Carcinogenesis. Mechanisms of Action. Edited by G. E. W. WOLSTENHOLME AND MAEVE O'CONNOR. 336 pp., $9.50. Little, Brown & Co., Boston, Mass.

Ciba Foundation Symposium. Regulation of Cell Metabolism. Edited by G. E. W. WOLSTENHOLME AND CECILIA M. O'CONNOR. 387 pp., $9.50. Little, Brown & Co., Boston, Mass.

Molecules and Mental Health. Edited by FREDERIC A. GIBBS. 189 pp., $4.75. J. B. Lippincott Co., Philadelphia, Pa.

Moloy's Evaluation of the Pelvis in Obstetrics, 2nd edition. By CHARLES M. STEER. 131 pp., $4.00. W. B. Saunders Co., Philadelphia, Pa.

The Mouth. Its Clinical Appraisal. By A. B. RIFFLE. 118 pp., $3.50. J. B. Lippincott Co., Philadelphia, Pa.

Neuropharmacology. Transactions of the Fourth Conference, Sept. 1957. Edited by HAROLD A. ABRAMSON. 285 pp., $5.00. The Josiah Macy, Jr. Foundation, New York, N. Y.

Physiology of Prematurity. Transactions of the Third Conference, March, 1958. Edited by JONATHAN T. LANMAN. 157 pp., $3.00. The Josiah Macy, Jr. Foundation, New York, N. Y.

Radiation Therapy. By WALTER T. MURPHY. 1041 pp., $25.00. W. B. Saunders Co., Philadelphia, Pa.

HEREDITARY ASPECTS OF EPILEPSY

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There is considerable diversity of opinion in the literature (1) concerning whether or not epilepsy is inherited. Various authors classify the different types of epileptic seizures in different ways. The use of different schemes of classification may be partially responsible for the lack of agreement on inheritance of epilepsy. Another factor which may be responsible for the difference of opinions is that few studies employed adequate controls to determine the frequency of epilepsy in the general population.

In this study we have used the classification of epileptic seizures which is given below. We have compared the various groups of epileptic patients with a control population which we believe to be comparable to our patient group in all significant factors.

CLASSIFICATION AND DEFINITIONS

A. Classification of Epileptic Seizures

1) Major motor epilepsy

Generalized convulsions, excluding simple febrile convulsions, breath-holding spells, and metabolically caused convulsions (such as hypoglycemic). Major motor epilepsy is further divided into:

a) *Secondary* or *organic major motor epilepsy.* Epilepsy associated with brain damage, as evidenced by findings (other than convulsions) existing prior to or at the time of onset of convulsions. In our study, convulsions associated with cerebral degenerative diseases have been excluded.

b) *Idiopathic major motor epilepsy.* Epilepsy where no evidence of organic brain damage was found.

2) Petit mal epilepsy

Spells which consist essentially of transient lapses of consciousness associated with bilaterally synchronous three per second spike and wave forms in the electroencephalogram. (Transient lapses of consciousness which frequently appear after treatment of major motor epilepsy, and which are not associated with three per second spike and wave forms in the electroencephalogram, are classified as "abortive major motor seizures.")

3) Minor motor epilepsy

Akinetic, massive myoclonic and head-nodding spells of short duration. Although these are often considered as part of the "petit mal triad" we consider that the clinical differences between these spells and petit mal epilepsy warrant placing them in a separate group.

4) Psychomotor epilepsy

Bizarre and variable types of seizures characterized by abnormal and inappropriate behavior associated with some clouding of consciousness and amnesia for the event.

5) Focal epilepsy

Convulsions which involve only part of the body. In this study no distinction is made between convulsions which are entirely focal in nature and convulsions which are sometimes focal and sometimes generalized.

6) Mixed epilepsy

Any combination of two or more of the above types.

B. Definitions of Terms used with a Special Meaning in This Paper

1) Simple febrile convulsions

Generalized seizures of short duration occurring during the first four or five years of life soon after the onset of an elevation of temperature. When these patients are afebrile they have normal electroencephalograms. (Patients who have seizures associated with an elevation of temperature who do not present all of the above findings are classified as having "epilepsy precipitated by fever.")

2) Prevalence of epilepsy among family members

The per cent of family members who have had convulsions at any time in their lives.

3) Close relative

Parent, sibling, maternal half sibling, (i.e. half-sibling of proband through mother) or child. (Paternal half siblings were excluded from the study.)

4) Statistically significant, significant difference, etc.

The probability of a difference of this magnitude being due to chance is 5 per cent or less. (P less than .05)

5) Age

All ages are stated to the nearest birthday. Thus "3 years" for example, includes all ages from 2½ years to 3½ years.

MATERIAL AND METHODS

This study was conducted by interviewing a group of epileptic patients or, in the case of children, a responsible adult member of the patient's family, and by interviewing in a similar fashion a group of non-epileptic patients. Results of the interviews were put on punch cards and analyzed by the usual methods.

All interviews were done by the same observer (V.E.) and an attempt was made to ask all of the questions in the same manner and without indicating what answer was desired. Unfortunately the observer always knew whether he was interviewing a test family or a control family. Test families were told that they were "cooperating in a study to find out some of the causes of epilepsy." Control families were told that the hospital was "conducting a survey to find out how many people had certain illnesses," and epilepsy was only mentioned after they had agreed to be interviewed. A negligible number of patients refused to be

interviewed or refused to answer all of the questions, and these few cases were distributed equally between the control and test groups.

The test group was drawn from patients attending the seizure clinics of The Johns Hopkins Hospital and from private patients of one of the authors. These private patients were nearly all attracted to us by our connection with the hospital, and we believe they were drawn from the same population as private patients attending other clinics in The Johns Hopkins Hospital. An attempt was made to interview every patient who attended these clinics over a two year period: we believe that over 80 per cent were included. Patients who were not included were for the most part those who attended the clinics during the first year of the study when it was impossible to interview as many patients as arrived each day, but who ceased coming to the clinics before the interviews were terminated. No patient was interviewed on his first clinic visit.

Control patients were drawn from other chronic disease clinics of The Johns Hopkins Hospital. At the start of a clinic, the clinic secretary's list was consulted for names of patients not previously interviewed. The secretary then introduced the interviewer to as many of these patients as time permitted, and the patient was interviewed. There was no selection of patients until the end of the study, when fifty children in one clinic were selected on the basis of age (under 5 years) in order to make the control group more nearly comparable to the test group in age.

In the event that more than one member of a family attended the clinics, each member was considered as a separate proband. Very few such instances of multiple probands were found.

Foster children and adopted children (or adults) were excluded from both groups. Patients who had had a convulsion or seizure of any type were excluded from the control group. When making up the test group, patients with an incomplete diagnosis, or with a diagnosis (such as hysteria) which did not include epilepsy, were excluded. All diagnoses of epilepsy were made by the authors.

For affected relatives, diagnoses were generally made by the interviewer based on the best description obtainable. In a few cases it was possible to check hospital records for these diagnoses, but for the most part they were made at second hand. It is felt that misclassifications may have been made in distinguishing between febrile convulsions and epilepsy in infants, and between idiopathic and organic major motor epilepsy.

Patients in the test group were divided into subgroups depending only on the clinical diagnosis of the type of epilepsy. The number of affected relatives was not considered in this division. The following subgroups were formed:

1. Organic major motor epilepsy
2. Idiopathic major motor epilepsy
3. Focal epilepsy (with or without major motor spells)
4. Petit mal epilepsy
5. Minor motor epilepsy
6. Psychomotor epilepsy
7. Mixed epilepsy
   a. Major motor + minor motor
   b. Major motor + petit mal
   c. Major motor + psychomotor

For reasons which will be stated later, the idiopathic major motor group was further subdivided into groups with onset from 0 to 3 years, 4 to 9 years, 10 to 15 years, and 16 years or over.

Other types of epilepsy than the ones listed occurred too infrequently in our clinic to make

statistical analysis worthwhile. In particular, simple febrile convulsions, although they are among the common seizures of childhood, are now treated in our general pediatric clinic, and are no longer seen in the seizure clinics.

A total of 669 patients with 3362 close relatives were included in the test series, and 470 patients with 2858 close relatives were included in the control series. Further breakdowns will be given below.

RESULTS

A. The Control Series

Probands for the control series were drawn from four sources.

1. The rheumatic heart disease clinics of the Johns Hopkins Hospital. (226 cases.) These patients included children and adults, both white and non-white. They were drawn almost entirely from the economic group with family incomes below $5000 per year.
2. The Hearing and Speech clinic of the Harriet Lane Home. (163 cases.) These patients were also of all ages. There was a preponderance of white patients. Nearly all of the control families with incomes over $5000 per year came from this group, as this clinic is a unique facility providing services unobtainable elsewhere in this community and many patients are referred to it by their own doctors. We believe that the patients from this clinic who were drawn from upper economic groups are comparable to the private patients included in the test series.
3. The Diabetes Clinic of the Harriet Lane Home. (29 cases.) This group included white and non-white children up to 14 years of age, all from families of low income.
4. The Ear, Nose, and Throat Clinic of the Johns Hopkins Hospital (52 cases.) Only children under 5 years of age were included in the group from this clinic. White and non-white infants and children from families with incomes below $5000 were included.

This selection of control patients makes it difficult to obtain all types of comparisons between the test and control groups for the following reasons: The Hearing and Speech Clinic treats patients with speech problems due to brain injury. For this reason, comparisons of birth factors which might cause brain injury cannot be made. This group also contains many patients with cleft palates, and because of this, comparisons of factors relating to congenital anomalies cannot be made. The control series differs in other respects also from "comparable normal controls." Therefore it was decided to consider only the incidence of convulsions among relatives of the control series.

The comparison of the groups comprising the control series is shown in Tables Ia and Ib. No significant differences can be demonstrated between these groups in respect to the incidence of seizures among close relatives. The "Diabetes + ENT" shows a higher per cent of convulsions among relatives than the "Hearing and Speech" or "Rheumatic Fever" groups. Although this might be explained by the age differences of the probands and their relatives, the finding is not statistically significant. No important bias is introduced by combining these groups into a single control series.

Factors such as aggregation of birth injuries into certain economic groups

TABLE Ia

Comparison of Sources of Control Probands. Convulsions of All Types Among Close Relatives. No Significant Differences Are Demonstrable.

Source of Probands	Number of Probands	Number of Close Relatives (n)	Per Cent of Relatives with Convulsions of Any Type (p)	Standard Error of p (SE p)	95% Confidence Limits of p (CL 95)
Rheumatic Clinics	226	1588	2.96	0.43	2.10–3.82
Hearing and Speech Clinics	163	926	2.27	0.48	1.31–3.23
Diabetes + ENT	81	344	3.49	0.99	1.51–5.47
Total	470	2858	2.80	0.31	2.18–3.42

TABLE Ib

Comparison of Sources of Control Probands. Major Motor Convulsions Among Close Relatives. No Significant Differences Are Demonstrable.

Source of Probands	Number of Probands	Number of Close Relatives (n)	Per Cent of Relatives with Major Motor Convulsions (p)	Standard Error, of p (SE p)	95% Confidence Limits of p (CL 95)
Rheumatic Clinics	226	1588	1.70	0.32	1.06–2.34
Hearing and Speech Clinics	163	926	1.51	0.40	0.71–2.31
Diabetes + ENT	81	344	2.33	0.81	0.71–3.95
Total	470	2858	1.72	0.24	1.24–2.20

TABLE IIa

Comparison of Controls by Race and Income. Convulsions of All Types Among Close Relatives. No Significant Differences are Demonstrable.

Race	Income	Number of Probands	Number of Close Relatives (n)	Per Cent of Relatives with Convulsions of Any Type (p)	Standard Error of p (SE p)	95% Confidence Limits of p (CL 95)
White	Less than $5000/yr	195	1178	3.06	0.50	2.06–4.06
	Over $5000/yr	87	423	2.84	0.81	1.22–4.46
Non-white	Less than $5000/yr	175	1162	2.75	0.48	1.79–3.71
	Over $5000/yr	13	95	2.11	1.47	0–5.05
Total White		282	1601	2.87	0.42	2.03–3.71
Total Non-White		188	1257	2.71	0.46	1.79–3.63
Total less than $5000/yr		370	2340	2.82	0.34	2.14–3.50
Total over $5000/yr		100	518	2.70	0.71	1.28–4.12
Total		470	2858	2.80	0.31	2.18–3.42

TABLE IIb

Comparison of Controls by Race and Income. Major Motor Convulsions Among Close Relatives. No Significant Differences are Demonstrable.

Race	Income	Number of Probands	Number of Close Relatives (n)	Per Cent of Relatives with Major Motor Convulsions (p)	Standard Error of p (SE p)	95% Confidence Limits of p (CL 95)
White	Less than $5000/yr	195	1178	1.70	0.38	0.94–2.46
	Over $5000/yr	87	423	1.42	0.58	0.26–2.58
Non-White	Less than $5000/yr	175	1162	1.89	0.40	1.09–2.69
	Over $5000/yr	13	95	2.11	1.47	0–5.05
Total, White		282	1601	1.62	0.31	1.00–2.24
Total, Non-white		188	1257	1.91	0.39	1.13–2.69
Total, Less than $5000/yr		370	2340	1.79	0.27	1.25–2.33
Total, Over $5000/yr		100	518	1.54	0.54	0.46–2.62
Total		470	2858	1.72	0.24	1.24–2.20

TABLE IIIa

Comparison by Age of Control Probands. Convulsions of All Types Among Close Relatives. Significant Differences are Present.

Age of Proband	Number of Probands	Number of Relatives (n)	Per Cent of Relatives with Convulsions of Any Type (p)	Standard Error of p (SE p)
0–8	146	632	3.16	0.70
9–19	194	1073	3.45	0.56
20–39	70	598	1.84	0.55
40+	60	555	2.16	0.62
Total	470	2858	2.80	0.31

TABLE IIIb

Comparison by Age of Relatives of Control Probands. Significant Differences are Present.

Age of Relative	Number of Relatives (n)	Per Cent of Relatives with Convulsions of Any Type (p_1)	Per Cent of Relatives with Major Motor Convulsions (p_2)	Standard Error of p_1 (SE p_1)	Standard Error of p_2 (SE p_2)
0–8	623	3.05	2.09	0.69	0.57
9–19	529	3.02	1.13	0.75	0.46
20–39	936	3.42	2.24	0.59	0.49
40+	727	1.51	1.24	0.45	0.41
Unknown	43	0	0		
Total	2858	2.80	1.72	0.31	0.24

or the difficulty of epileptics in finding employment would lead one to expect a higher prevalence of epilepsy among people with lower incomes. Tables IIa and IIb show that this was not true for the control group, as no significant differences in the prevalences of the epilepsy could be demonstrated between white and non-white or between high and low income families in this series.

Age differences, however, have a small, but statistically significant effect. Tabe IIIa shows significant differences in the prevalences of epilepsy when the control group is divided by the age of the probands, and Table IIIb demonstrates that this probably depends in part on the ages of the proband's relatives. There is reason to believe that it may also depend in part on reporting error: relatives 40 years of age and over were reported to have a significantly lower prevalence of seizures than those aged 39 years or less. This seems unlikely in view of the fact that any seizures at any age should have been included.

The currently quoted figure for the prevalence of epilepsy in the "general population" is 0.5 per cent. Our estimates reveal a prevalence between 2.18 per cent and 3.42 per cent among relatives of our control probands. The differences between our figures and the currently quoted ones may be explained in part by reporting errors: in many patients seizures do not recur beyond infancy or childhood, and are forgotten later.

B. Studies of Familial Aggregation of the Various Types of Epilepsy

In order to determine if the various test groups differed from the control group in prevalence of convulsions among relatives, it was decided to adjust the groups to standard populations which would contain equal numbers of each category of relatives (parent, etc.) and of each age group of relatives. This method eliminates the differences shown in Table III which depend on the age of the relative. It was considered unnecessary to adjust for socioeconomic status for a first approximation.

1.) Major motor epilepsy. (404 probands, 2127 close relatives.)

Patients with major motor epilepsy were divided by the criteria stated previously into "idiopathic major motor epilepsy" (321 probands, 1658 close relatives) and "organic major motor epilepsy." (83 probands, 469 close relatives.) Table IV shows the adjusted test and control prevalences of major motor convulsions and of all types of convulsions in these groups.[1]

No significant differences were found between the organic epilepsy group and the controls. The idiopathic epilepsy group showed significant evidence of familial aggregation of major motor convulsions, but not of seizures of other types.

[1] In order to simplify calculations, s was calculated by the formula $S = \sqrt{pq/n}$. Where age differences appeared greatest, the more correct method of calculating the variance for each cell was applied as a check. It did not materially change the results.

TABLE IV

Idiopathic and Organic Major Motor Epilepsy. Comparison with Control Series. Idiopathic Epilepsy Shows Significant Familial Aggregation of Major Motor Convulsions, but Not of Other Types. Familial Aggregation Cannot be Demonstrated in Organic Epilepsy. Adjusted for Relationship and Age of Relatives.

Type of Convulsion Relative	Type of Major Motor Convulsion Proband	No. of Cases	No. of Relatives (n)	Adjusted Per Cent of Relatives with Convulsion, Test Group (p_t)	Adjusted Per Cent of Relatives with Convulsions, Control (p_c)	Standard Error of Difference (SE_{diff})	Probability That This Large a Difference Could Arise by Chance (P)
All types	Idiopathic	321	1658	6.53	2.78	0.70	<.0005
	Organic	83	469	2.24	2.88	0.72	.37
Major motor	Idiopathic	321	1658	5.26	1.75	0.62	<.0005
	Organic	83	469	1.79	1.79	.65	1.0
Other	Idiopathic	321	1658	1.27	1.03	0.33	.47
	Organic	83	469	0.45	1.09	0.42	.13

TABLE V

Idiopathic Major Motor Epilepsy, by Age of Onset of Epilepsy. Comparison with Control Series. Adjusted for Relationship and Age of Relatives.

Age of Proband at Onset of Epilepsy	No. of Cases	No. of Relatives (n)	Adjusted Per Cent of Relatives with Major Motor Epilepsy, Test Group (p_t)	Adjusted Per Cent of Relatives with Major Motor Epilepsy, Control Group (p_c)	Standard Error of Difference (SE_{diff})	Probability That This Large a Difference Could Arise by Chance (P)
0–3	136	622	8.29	2.17	1.11	<.0005
4–9	85	390	4.26	1.76	1.06	.02
10–15	66	382	5.75	1.74	1.22	.0015
16+	34	264	3.36	1.56	1.10	.10

It is well known (2) that the prevalence of convulsions among relatives varies with the age of onset of major motor epilepsy. Ounsted (3) has reported that the highest prevalence is found when the proband's epilepsy started before 3½ years of age. We have been impressed with the frequency with which epilepsy starts in early adolescence, and have a clinical impression that this type of epilepsy may differ from epilepsy starting in childhood. When we examined familial prevalence figures broken down by age of onset of the proband's epilepsy, we were impressed with apparent differences in the prevalences and grouped our cases as shown in Table V, into groups with onset from 0 to 3 years, 4 to 9 years, 10 to 15 years, and 16+ years. We realize that these differences are partially subjective and are emphasized by the choice of ages at which we made the divisions.

The most striking familial aggregation of major motor convulsions was found in the group with onset from 0 to 3 years. The group with onset from 10 to 15 years showed a higher prevalence of major motor convulsions than did the

group with onset from 4 to 9 years. No evidence was found for familial aggregation of epilepsy when the proband's seizures started at 16 or more years of age, but the small numbers in this group make it impossible to say that some familial aggregation does not exist.

While the differences from the control group are apparent, the differences between the three groups in which familial aggregation can be demonstrated are not statistically significant. It is not possible to state that these age groupings represent different types of epilepsy. There is an apparent tendency for the relatives in these groups to have epilepsy which starts at the same age as the proband's. (Table VI.) When corrections are made for the age distributions of the relatives by the method of Harris (4) and the results adjusted for the total number of cases in each proband group, the observed ages of onset of the relatives do not differ significantly from the expected distribution.

Our initial approximation indicates that there is familial aggregation of idiopathic major motor epilepsy, and that this aggregation appears when the epilepsy starts before 15½ years of age. In order to rule out socioeconomic factors as a cause of this aggregation, the various groups of probands were matched proband by proband with members of the control series who were of the same age, sex, and race, and who had similar family incomes (above or below $5000 per year.) These matched groups were then compared.[2] (Table VII.) The results are similar to those in the unmatched groups, in each case except idiopathic major motor epilepsy starting from 4 to 9 years of age. In this one group the difference between the test and control groups remains, but the difference is no longer statistically significant. It is believed that this is only a reflection of the statistical uncertainty caused by using a small control group, and that the tendency for familial aggregation is a real one which is too small to be shown with the number of relatives in these groups.

The idiopathic major motor epilepsy group was sub-divided by other criteria in order to see if these criteria would separate subgroups which differed in the familial incidence of convulsions. Sex, economic level of home address, occupation of head of family, race and income, presence or absence of mental retardation in the proband, and the electroencephalographic findings all have no demonstrated bearing on the prevalence of convulsions among family members (Table VIII.)(Factors relating to birth injury will be considered below.)

An apparent difference was found when the group was broken down according to whether the proband had ever had convulsions associated with a high fever, (i.e. epilepsy precipitated by fever. No simple febrile convulsions were included in this group.) This difference was due to the early age at which convulsions are usually precipitated by fever; it disappeared when adjustment was

[2] The test of significance did not assume that the matching introduced a correlation, as the probands were matched, not the relatives. This is the more conservative method.

TABLE VI

Comparison of Observed and Expected Ages of Onset of Major Motor Epilepsy: in Relatives of Proband with Idiopathic Major Motor Epilepsy. (Relatives with Unknown Age of Onset Excluded.) The Differences are not Significant.

Age of Onset, Proband		Age of Onset, Relative			
		0-9	10-19	20+	Total
0–3	Observed	33	4	4	41
	Expected	28.98	6.16	5.86	41.00
4–9	Observed	7	3	3	13
	Expected	8.56	2.06	2.38	13.00
10–15	Observed	8	4	5	17
	Expected	10.78	2.85	3.37	17.00
16+	Observed	1	1	2	4
	Expected	2.30	0.68	1.02	4.00
Total	Observed	49	12	14	75
	Expected	50.62	11.75	12.63	75.00

TABLE VII

Major Motor Epilepsy. Comparison with Control Group Matched by Proband for Age, Sex, Race, and Income

Type of Epilepsy	Number of Probands in Matching Groups	Number of Relatives in Test Group (n_t)	Test Group		Control Group		Standard Error of Difference (SE_{diff})	Probability of Difference Arising by Chance (P_{diff})
			Adjusted per cent of relatives with major motor convulsions (p_t)	Standard error of p_t ($SE\ p_t$)	Adjusted per cent of relatives with major motor convulsions (p_c)	Standard error of p_c ($SE p_c$)		
Organic major motor	68*	386	2.16	0.80	1.98	0.50	0.94	.85
Idiopathic major motor, all ages of onset	273	1402	5.75	0.62	1.50	0.30	1.14	<.0005
Idiopathic major motor, onset from 0 to 3 yrs.	125	565	7.39	1.06	1.45	0.48	1.16	<.0005
Idiopathic major motor, onset from 4 to 9 yrs.	68	297	4.66	1.31	1.96	0.73	1.50	.072
Idiopathic major motor, onset from 10 to 15 yrs.	54	326	6.07	1.29	2.05	0.72	1.48	.01
Idiopathic major motor, onset at 16 or more yrs.	26	214	2.35	1.49	0	0.86	1.71	.16

* Control group contained 2 probands for each test proband. Number given is for test group. In other series, each test proband was matched with one control proband.

made by age of onset of the epilepsy. (P of difference = .30) Age adjustment did not materially change the results given in Table VIII.

There was no relation between electroencephalographic findings and the prevalence of convulsions among relatives. In addition to presence or absence

TABLE VIII

Division of Idiopathic Major Motor Epilepsy Group by Various Criteria. None of These Differences is Significant.

Criterion	Division	Number of Probands	Number of Close Relatives	Per Cent of Close Relatives with Major Motor Convulsions
Sex	Male	142	719	5.52
	Female	131	683	6.00
Economic level of home address	Upper 3/10	55	308	6.8
	Middle 5/10	64	325	7.7
	Lower 3/10	24	129	4.6
	Suburbs	61	280	6.1
	Outside of metropolitan area	69	360	3.0
Occupation of head of family	"White collar" & professional	78	344	5.23
	Skilled labor	94	490	4.89
	Unskilled labor	68	364	6.31
	Other, and not recorded	33	204	7.35
Mental retardation	Present	43	226	6.19
	Absent	230	1176	7.23
Electroencephalographic findings	Normal or not done	106	574	5.40
	Generalized abnormalities	85	434	5.29
	Focal or lateralized abnormalities	82	394	6.59
Race and income	White, under $5000/yr	128	647	5.10
	White, over $5000/yr	89	499	7.01
	Non-white	56	256	4.74

of generalized electroencephalographic abnormalities, we examined specific abnormalities and various combinations of them. No significant differences were found.

2.) *Focal epilepsy.* (89 probands, 466 close relatives.)

No cases of focal epilepsy were found among the close relatives of this group. No significant differences were found upon comparison with the control group either in the number of relatives with seizures of any type or the number of relatives with major motor convulsions. Results in this group are included in Table IX.

3.) *Petit mal epilepsy* (34 probands, 130 close relatives.)

No cases of petit mal epilepsy were found among the close relatives of this group. No significant differences were found upon comparison with the control group either in the number of relatives with seizures of any type or in the number of relatives with major motor convulsions. Results are included in Table IX.

4.) *Minor motor epilepsy.* (31 probands, 120 close relatives.) No cases of minor

TABLE IX

Different Types of Epilepsy Compared with Controls for Incidence of Convulsions Among Close Relatives. Adjusted for Relationship and Ages of Relatives. Significant Differences in the Incidence of Major Motor Convulsions are Found in Mixed Epilepsy.

Type of Epilepsy	Test Group		Major Motor Convulsions				Convulsions of Any Type			
	Number of probands	Number of close relatives	% affected relatives (p)		Standard error of difference	P of difference	% affected relatives. (p)		Standard error of difference	P of difference
			test	contr.			test	contr.		
Focal	89	466	2.17	1.75	0.75	.59	2.70	2.77	0.85	.93
Petit mal	34	130	0.66	1.93	0.82	.12	1.37	3.07	1.28	.20
Minor motor	31	120	0.42	1.94	0.88	.10	1.22	3.10	1.47	.20
Psychomotor	11	61	0			—	0			—
Major motor + petit mal	30	135	3.95	1.75	1.79	.22	3.95	2.80	1.80	.52
Major motor + minor motor	42	189	4.60	1.95	1.49	.08	6.19	3.13	1.74	.08
Major motor + psychomotor	28	133	6.50	1.81	1.96	.02	6.50	2.85	1.97	.07
All mixed epilepsy	100	457	4.65	1.53	1.01	.002	5.24	2.69	1.05	.017

motor epilepsy were found among the close relatives of this group. No significant differences were found upon comparison with the control group either in the number of relatives with seizures of any type or in the number of relatives with major motor convulsions. Results are included in Table IX.

5.) *Psychomotor epilepsy.* (11 probands, 61 close relatives.)

None of the close relatives in this group had seizures. Results are included in Table IX.

6.) *Mixed epilepsy.* (100 probands, 457 close relatives.)

As is shown in Table IX, there is a significantly higher prevalence of major motor convulsions among relatives in this group than in the control group. Other types of seizures showed no significant differences from the control prevalences.

a.) *Major motor + petit mal* (30 probands, 135 close relatives.) No cases of petit mal epilepsy were found among the close relatives of this group. Although the prevalence of major motor spells was higher than the control, the difference was not significant. (Table IX.)

b.) *Major motor + minor motor* (42 probands, 189 close relatives.) Two cases of minor motor epilepsy were found among the 189 close relatives in this group. This is not significantly different from the control series. (There were no cases of minor motor epilepsy among 2203 relatives in the control series.) The inci-

dence of major motor spells was higher than the control series: this difference was not significant. (Table IX.)

When the above two groups are combined, the difference in the incidence of major motor spells is still not significantly different from the control series. (P = .08)

c.) Major motor + psychomotor. (28 probands, 133 close relatives.)

Two cases of psychomotor epilepsy were found among the 133 close relatives of this series. This was one of the few instances of multiple probands from the same family: the two cases were related to each other and each was also a proband. If both are counted, the prevalence of psychomotor epilepsy in the group is still not significantly different from the control group, where 2 cases of psychomotor epilepsy were found among 2577 close relatives.

The prevalence of major motor convulsions was significantly higher than in the control group. This group apparently accounts for most of the familial aggregation of major motor epilepsy found in the "mixed epilepsy" group.

C. Tests of Genetic Hypotheses

Demonstration of familial aggregation of a trait is not a complete proof of inheritance. Genetic factors are not the only possible causes of familial aggregation, as environmental causes of a condition may also be more prevalent in certain families. For this reason we have tested our data against some mathematical models of genetic transmission. The various means of genetic transmission of a trait may be divided into single and multiple gene transmission. A gene may be dominant, that is, express itself in the heterozygote; or it may be recessive, and only find phenotypic expression in the homozygote. In addition, it may have incomplete penetrance, and express itself in less than 100 per cent of the genotypes which carry the trait.

1.) Single dominant gene hypothesis

This hypothesis requires that at least 50 per cent of the children of an affected individual should demonstrate the trait. Assuming full penetrance, the only mechanism by which an affected individual could arise from two unaffected parents would be mutation.

These and other conditions are not met in our series. The vast majority of our patients came from unaffected parents, and a mutation rate large enough to account for this would in inconceivably greater than any yet measured for traits known to be genetic. Only about 4½ per cent of the children of the probands with idiopathic major motor epilepsy starting from 0 to 15 years of age had major motor seizures (4 out of 88). It may be concluded that we are not dealing with a dominant gene with full penetrance.

2.) Single recessive gene hypothesis

The hypothesis states that an affected individual acquired one affected gene from each parent. Either parent could be a heterozygote or a homozygote. If two affected individuals mate, all of the offspring would be affected; if an affected individual mates with an unaffected one, the offspring will all be unaffected heterozygotes if the unaffected parent does not carry the gene, or they will be 50 per cent unaffected heterozygotes and 50 per cent affected homozygotes if the unaffected parent does carry the gene. The mathematical model of this situation must make allowances for the proportion of heterozygotes who will not be found because they do not show the trait and do not have children who show it.

a.) "The Binomial Hypothesis" This hypothesis states that among affected individuals, the proportion of parental matings in which two, one, or no parents are affected will be p^2, 2pq, and q^2, where p^2 is the proportion of affected individuals in the population, $p + q = 1$, and $p =$ the gene frequency.

In the group of idiopathic major motor epilepsy starting from 0 to 15 years of age, the number of cases arising from 2, 1, and no affected parents were 1, 45, and 241 respectively. This is significantly different from the expected numbers of 5.7, 68.9, and 212.4, where the value of p^2 was calculated from the incidence of major motor epilepsy with this age of onset in the control population.

When the group was divided into cases with onset from 0 to 3, 4 to 9, and 10 to 15 years of age, similar significant differences from the expected values were found for each of the subgroups.

No significant difference from expected values was found when the test was applied to the subgroup with age of onset from 0 to 3 years and the number of parents with epilepsy starting from 0 to 15 years was counted. (P more than 30 per cent.) In this case, the lowest of the several appropriate values of p^2 was chosen, in order to make the fit better. Similarly, the data could be made to fit the hypothesis when the test was applied to probands with onset from 10 to 15 years and parents with onset from 0 to 15 years.

b.) Test by number of consanguineous matings. It can be shown mathematically that a rare recessive trait is more likely to occur as the result of consanguineous matings than as a result of non-consanguineous ones. The converse is also true, that the matings which produce offspring showing such a trait are more likely to be consanguineous than are all other matings in a population. If the incidence of a trait and the proportion of consanguineous matings in the population are known, the expected number of consanguineous matings among the parents of those showing the trait can be calculated.

The frequency of first cousin marriages in the control population was 0.85 per cent. The expected frequency for the parents of probands with idiopathic major motor epilepsy starting before 15½ years of age was calculated to be at

least 1.32 per cent while the observed frequency was 0.70 per cent. This is significantly different from the expected frequency. When values were calculated for the various age groupings, the observed values were in every case significantly lower than the expected ones.

The significant difference in each of these cases depends on the relatively high value of 0.85 per cent obtained in the control population. This is much higher than other studies (5, 6) have shown in the United States, and comparable to recent figures from Copenhagen and France (7). There are no satisfactory estimates of consanguineity among United States urban negroes, who formed a large part of our group. Another large part of our group consisted of recent white immigrants from isolated mountain communities in West Virginia. It is our feeling that the consanguineity rate we obtained for our control population is comparable to that which would be expected in our test population. It is possible, however, that the control population was biased by drawing it from hospital outpatients. These might be expected to carry more injurious recessive traits than the general population. Thus hospital outpatients might be expected to arise from consanguineous matings more frequently than the general population. If the correct "general population" consanguineity rate for these calculations were as low as 0.2 per cent the results would be entirely compatible with a recessive gene hypothesis.

c.) ***Haldane's test of recessive inheritance.*** Haldane (8) pointed out that in the situation of incomplete ascertainment of affected individuals in a population, with multiple selection (i.e. more than one affected child can be independently ascertained in a family) the methods used which assume complete ascertainment will give a high value for the probability of an affected child. The methods which assume incomplete ascertainment with single selection will give a low value. The true value will be below the one and above the other.

Our series was collected by methods which fit the criteria of incomplete ascertainment, as all of the epileptics in the population were not included, and of multiple selection, as both of two siblings attending the clinic were included as probands. (Excluding the "second" sibling raises other problems, and does not simplify the calculation.)

The test which assumes complete ascertainment (the "a priori" "proband," or "direct" method) consists of dividing the families into those with no, 1 or 2 affected parents, and further dividing by family size. For each size family, a factor can be derived for the number of siblings expected to show the trait. The sum of the expected numbers can be compared with the observed numbers of affected children in the families.

For the group of idiopathic major motor epilepsy with onset from 0 to 15 years, the expected number of affected children was 265.9 for the families with no affected parents, and 53.6 for those with one affected parent.

The observed numbers were 249 and 50. The first of these figures is signifi-

cantly lower than the expected number. The second is not significantly different, but this may well be due to the small numbers involved, as the standard error was 2.93, which means that any difference less than 6 would not be significant.

When the subgroups were tested, significant differences were found in all the groupings with no parents affected. No significant difference was found in the group with onset between 4 and 9 years with one affected parent. This again may well be due to the small number of individuals in the series.

In making the above calculations, all unaffected siblings who had not passed through the ages of onset under consideration were eliminated from the calculation. This would have the effect of artificially lowering the expected numbers. As the observed numbers were lower than the expected ones in spite of this, it may be concluded that the proportions of affected siblings in the groups tested is lower than would be expected with a recessive gene hypothesis.

3.) Reduced penetrance hypothesis

We have not attempted to fit our data to this hypothesis. It is probable that one could select factors for the amount of penetrance, number of genes, or lethal effect of homozygosity which would fit any example of familial aggregation as long as the numbers affected were lower than would be expected with a completely penetrant single gene. Such calculations may be made only when the genetic nature of the phenomenon has already been proven.

4.) Proof of inheritance by exclusion of other possibilities

It is generally agreed that epilepsy may be caused by brain damage, and that it is possible for an infant's brain to be damaged by trauma occurring before or during, as well as after birth. In the presence of this possibility, it may not be assumed that the observed familial aggregation is due solely to genetic factors unless it can be shown that perinatal injury bears no relation to familial aggregation of epilepsy. This will be considered in the next section.

D. Tests of Non-genetic Hypothesis

It may be assumed that if a given event in the perinatal period is the cause of the familial aggregation observed in major motor epilepsy, then if the families of those probands who experienced the event are grouped together, these families will show a higher prevalence of epilepsy than the remaining families.

Table X shows the results of grouping families according to various criteria which might be expected to separate out a "birth injury" group. No significant differences were found when the families were divided according to the proband's length of gestation, type of delivery, type of anesthesia used at the delivery, length of labor, cyanosis noted at birth, or whether the proband's mother had ever had miscarriages. In addition, no significant differences could

TABLE X

Division of Idiopathic Major Motor Epilepsy Group by Criteria Relating to Birth Injury. None of These Differences is Significant.

Criterion	Division	Number of Probands	Number of Close Relatives	Per Cent of Close Relatives with Major Motor Convulsions
Gestation	Normal	237	1182	5.84
	Premature	16	94	3.19
	Postmature	5	23	8.69
	Unknown	15	103	5.83
Tye of delivery	Vertex without forceps	167	842	5.22
	Other	61	239	7.53
	Unknown	45	321	5.61
Type of anesthesia	None, local, or regional	120	600	4.83
	General	95	384	6.51
	Unknown	58	418	6.22
Length of labor, proband first live born child	Over 12 hours	29	80	11.25
	Under 12 hours	35	109	6.42
	Unknown	27	126	10.31
Length of labor, proband not first live born child	Over 12 hours	16	88	3.40
	Over 6 hours	39	201	5.97
	Under 12 hours	118	622	5.14
	Under 6 hours	95	509	4.51
	Unknown	48	367	4.35
Cyanosis noted at birth	Present	13	55	7.27
	Absent	231	1146	5.75
	Unknown	29	201	4.98
Miscarriages	Mother had pregnancies miscarry	68	317	5.04
	No known miscarriages	137	608	6.08

be demonstrated when divisions were made by age of onset of the proband's epilepsy and these criteria. Thus we are unable to demonstrate that any of these factors is a cause of the familial aggregation.

The situation is different when all other perinatal abnormalities are examined. These abnormalities are a diverse group, with too few probands displaying any one abnormality to enable a more specific breakdown. They include signs of fetal distress, signs or a history suggesting mechanical trauma during delivery (such as a history of holding the fetal head back to prevent delivery, or forceps marks noted after delivery), and maternal toxemia or hypertension. The effect of these factors is shown in Table XI. Families in which the probands showed one of these abnormalities had a significantly higher prevalence of major motor epilepsy among close relatives than families where no such ab-

TABLE XI

Effect of Miscellaneous Perinatal Abnormalities. Numbers are too Small to Permit a More Detailed Breakdown. The Differences in the Totals are Significant.

Abnormality	Age of Onset of Proband's Epilepsy	Number of Probands	Number of Relatives	Per Cent of Relatives with Major Motor Convulsions (p)	Standard Error of p
Fetal distress, difficult labor, signs of fetal injury, maternal toxemia or hypertension	0–3	14	47	21.3	5.96
	4–9	4	18	16.4	
	10–15	3	39	7.7	
Total		21	104	15.35	3.53
None recorded	0–3	111	518	6.18	1.06
	4–9	64	279	3.58	1.13
	10–15	51	284	5.94	1.40
Total		226	1081	5.45	0.69

TABLE XII

Per Cent Risk of Major Motor Epilepsy for Close Relatives of Proband with Idiopathic Major Motor Epilepsy

Age	Control	Age of Onset of Proband's Epilepsy			
		0–15½	0–3½	3½–9½	9½–15½
A. Cumulative Risk					
4½	0.66	3.22	5.60	0.81	1.92
9½	1.08	4.25	6.57	2.35	2.54
14½	1.22	5.06	7.05	2.68	4.26
19½	1.43	5.50	7.61	3.43	4.26
29½	1.96	6.54	8.66	4.78	5.14
39½	2.27	7.53	9.35	6.02	6.27
49½	2.43	8.21	—	7.10	7.07
Number of relatives	2787	1370	618	379	373
Number of relatives with major motor epilepsy	48	84	46	17	19
B. Incremental Risk					
0–4½	0.66	3.22	5.60	0.81	1.92
4½–9½	0.42	1.03	0.97	1.54	0.62
9½–14½	0.14	0.81	0.48	0.33	1.72
14½–19½	0.21	0.44	0.56	0.75	—
19½–29½	0.53	1.05	1.05	1.35	0.89
29½–39½	0.30	0.99	0.70	1.24	1.12
39½–49½	0.16	0.68	—	1.07	0.80

TABLE XIII

Per Cent Risk of Major Motor Epilepsy for Full Siblings of Proband with Idiopathic Major Motor Epilepsy

Age	Control	Age of Onset of Proband's Epilepsy			
		0–15½	0–3½	3½–9½	9½–15½
A. Cumulative Risk					
4½	0.66	3.14	4.80	1.99	1.64
9½	1.08	3.62	5.30	2.78	2.24
14½	1.22	4.72	6.09	2.78	4.38
19½	1.43	5.49	7.45	4.13	4.38
29½	1.96	7.21	—	7.86	5.58
39½	2.27	7.82	—	10.77	—
Number of siblings	2787*	647	301	158	188
Number of siblings with major motor epilepsy	48*	33	16	9	8
B. Incremental Risk					
0–4½	0.66	3.14	4.80	1.99	1.64
4½–9½	0.42	0.48	0.50	0.79	0.60
9½–14½	0.14	1.09	0.79	—	2.10
14½–19½	0.21	0.77	1.36	1.35	—
19½–29½	0.53	1.72	—	3.72	1.24
29½–39½	0.30	0.61	—	2.91	—

* All close relatives

normality was recorded. No further breakdown can be made, as the numbers are too small. It is of interest that the group in which such abnormalities were not found still has a higher incidence of major motor epilepsy among close relatives than the control.

E. Risk Tables

Regardless of the etiology of epilepsy, it is of value to know what the chances are of a given person developing it. Tables XII and XIII show the percentage risk of major motor epilepsy for close relatives, and for full siblings, of our patients with idiopathic major motor epilepsy starting before the age of 15½ years. The tables are divided into two parts: a cumulative risk table which shows the chance at birth of developing major motor epilepsy if the child survives to the age shown, and an incremental risk table which shows, for each 5 or 10 year age increment, the risk of developing major motor epilepsy during the period for an unaffected person who enters the period and survives to the end of it. We were unable to develop similar tables for children of our probands,

because of the small numbers involved, and for the same reason we are unable to show separate tables for siblings who had one affected parent versus siblings who had no affected parents. Our data tends to show that the risk is somewhat higher, however, if a parent is affected.

These risk tables may be used to estimate the chances of a given person developing epilepsy. The cumulative risk tables show the risk of epilepsy for a newborn. If the person under consideration has already passed a certain number of years without developing epilepsy, an estimate of future risk may be made by summing the risks of the appropriate years in the incremental risk tables.

These tables were constructed by standard life-table methods.[3]

DISCUSSION

A study such as the present one which is based on retrospective data obtained by the interview method must stand or fall on the accuracy of the information obtained at the interviews. In this study we were fortunate in obtaining excellent cooperation from our informants, and we felt at the time of interview that over 99 per cent of the informants were giving us information to the best of their ability. We believe that our data are a reasonably accurate reflection of the number of close relatives with convulsive disorders. It might be questioned whether certain types of seizures such as petit mal might have been present in relatives but not observed by anyone. However, each informant in the test series was intimately concerned with the proband, and was familiar with at least the type of epilepsy displayed by the proband. Only close family members were included in the study, and it may be presumed that unrecognized seizures did not exist to any great extent among relatives in the test series. It is reassuring that the prevalence of convulsions among relatives in the control series was higher than we had expected: the effect of unrecognized seizures, of course, would have been to lower the observed prevalences.

Two internal checks on the accuracy of information regarding seizures among family members were possible. In the group of idiopathic major motor epilepsy with onset from 10 to 15 years of age, our informants were divided between parents, where the patient was still young enough to be brought to the clinic by an older person, and the patients themselves. We could demonstrate no significant differences in the reported prevalences between the group in which

[3] If x = age; Ox = the number coming under observation at a given age x; Wx = the number lost to follow-up at age x, Nx and Dx equal respectively the number developing major motor epilepsy and dying at age x; then $qNx = Nx/Ox - \frac{1}{2}Wx$, and $qDx = Dx\ O^x - \frac{1}{2}Wx$. A survivorship curve was constructed by applying qNx and qDx to a standard population lx where $l_o = 100$: then n′x = lx times qNx, d′x = lx times qDx; and $l_{x+1} = lx - (n'x + d'x.)$ The cumulative risk tables were made by summing n′x. Risks are expressed in per cent.

a parent was the informant, and the group in which the proband was the informant. In addition, we found that the prevalence of convulsions among relatives decreased with increasing age of the probands. The difference disappeared when adjustment was made for age of onset of the proband's epilepsy.

The situation in regard to information concerning the proband's birth is quite different. The amount of information available from the probands themselves was negligible. Little information could be obtained from fathers. Even mothers would frequently report that they could remember little of what happened during the infant's birth. We did not consider negative reports in this area to be worth the trouble of analysis, although we feel that positive reports were probably accurate. We did not attempt to use birth information derived from the control group.

There is, in addition, a possible bias in this information. If there are several members of a family with epilepsy, it is possible that a mother might notice and remember events during the birth of her child more carefully than she would otherwise, in order to relieve herself of a feeling of guilt for having "bad heredity." It is not known whether such an effect exists, but it must be considered as a possible explanation for the familial aggregation of epilepsy which we found in our group with miscellaneous abnormalities in the perinatal period.

We were unable to demonstrate familial aggregation of epilepsy in any type of seizure except idiopathic major motor epilepsy. (It should be noted again that we did not include simple febrile convulsions in the study.) The types of epilepsy in which we found no evidence of familial aggregation were:

Organic major motor epilepsy
Focal epilepsy, with or without generalized seizures
Petit mal epilepsy
Minor motor epilepsy
Psychomotor epilepsy

In addition, we were unable to demonstrate familial aggregation in idiopathic major motor epilepsy when seizures started after 15½ years of age. The number of patients was small in this group, and it is not impossible that familial aggregation does occur, but to a degree that was too small for us to find. However, Alström (9) also failed to find familial aggregation in a large series of adult patients. In mixed epilepsy, we were unable to demonstrate familial aggregation of any type of epilepsy other than major motor. There is no evidence in the present study to show that any of these types of epilepsy is inherited.

Familial aggregation of major motor epilepsy can be demonstrated when the proband's epilepsy starts before 15½ years of age. The reasons for this aggregation are not clear, and they may be multiple. We have investigated several hypotheses, including genetic ones. We find no evidence that the aggregation is due to socioeconomic factors, although one might postulate that income and social level would be important factors in a disease associated with birth

trauma. We are unable to demonstrate that the aggregation follows any theoretic pattern of simple genetic inheritance. There is evidence that the aggregation may depend on familial aggregation of perinatal injury. But we pointed out above that this evidence rests on dubious data.

Other workers have also demonstrated familial aggregation of epilepsy. Lennox (10) showed prevalence figures among relatives of his probands which are comparable to ours. He also studied a large series of twins, and found a high concordance for epilepsy among monozygotic twins, and a much lower concordance among dizygotic twins. In this, his results were similar to the earlier series by Conrad (11) and Rosanoff, *et al.* (12). We cannot agree that inheritance is the only possible explanation for the combination of familial aggregation and high concordance among monozygotic twins. A familial tendency to an epileptogenic trauma occurring before or during birth might also explain the observations, and it has been well demonstrated by Lilienfeld and Pasamanick (13) that there is an association between perinatal injury and epilepsy. In addition, Yerushalmy and Sheerar (14) have shown that like-sexed twins show higher stillbirth, prematurity, and perinatal mortality rates than unlike-sexed twins. This may be interpreted to mean that perinatal injury is commoner in monozygotic than dizygotic twins. The causes of epilepsy may well be multiple, and present knowledge does not, in our opinion, allow one to rule out any of the possible causes.

The classification of epilepsy which we used in this study is a simple clinical one. It is not the only possible classification. Metrakos, *et al.* (15) have used one based on electroencephalographic abnormalities, and Ounsted (3) has included the response of the patient to treatment and other "follow-up data" in his classification. We have attempted to divide our "idiopathic major motor" epilepsy group by criteria which would separate groups with differing amounts of familial aggregation. None of the clinical criteria which we applied did this with the exception of the age of onset of the epilepsy. (We did not include response to treatment among the criteria which we examined.) Electroencephalographic criteria did not separate families with a high prevalence of seizures among relatives, nor did the association of fever with the convulsion, when simple febrile convulsions were excluded from the series. Nevertheless, we feel that our series is not a homogeneous one, and that there may well be types of epilepsy included in our classification of "idiopathic major motor" which are transmitted genetically. In addition, there may be types included which show familial aggregation because of perinatal injury. In this connection, it is of interest that 13 per cent of the pregnancies of the mothers of probands with idiopathic major motor epilepsy miscarried, compared to 9 per cent of the pregnancies of mothers in the control group. (Cases where the mother was not the informant were excluded.) This is a significant difference, although its meaning is not clear.

Our findings do not allow a definite answer to the question of whether epilepsy is inherited.

SUMMARY

Interviews were obtained with members of the families of 669 epileptics with 3362 close relatives, and 470 control patients with 2858 close relatives.

A small but significant familial aggregation of idiopathic major motor epilepsy was demonstrated. This aggregation appeared to vary with the age of onset of the proband's epilepsy, and was highest when the proband's epilepsy started between the ages of 0 and 3½ years, and 9½ and 15½ years. No familial aggregation could be demonstrated when the age of onset was over 15½ years, nor in other types of epilepsy.

Simple febrile convulsions were not included in the study.

Hereditary transmission of epilepsy could not be demonstrated, but it could not be ruled out. Familial aggregation of idiopathic major motor epilepsy may be due to familial aggregation of perinatal brain injury, to inheritance, or to a combination of these factors. Further studies of the prospective type are probably needed to clarify this, unless a test or trait more related to the "basic defect" can be found.

Risk tables are presented. The highest risk we found was to close relatives of probands who developed major motor epilepsy before 3½ years of age: 7.6 per cent of these relatives may be expected to develop major motor seizures by 19½ years of age, and 9.4 per cent by 39½ years of age. Comparable control risks were 1.4 per cent and 2.3 per cent.

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APPENDIX A

Age Distribution of Relatives of Probands with Idiopathic Major Motor Epilepsy

Relationship	Age	Onset 0–3			Onset 4–9			Onset 10–15			Onset 16+		
		Number	+ all spells	+ maj. mot. spells	Number	+ all spells	+ maj. mot. spells	Number	+ all spells	+ maj. mot. spells	Number	+ all spells	+ maj. mot. spells
Parent	9–19	2	0	0	0	0	0	0	0	0	0	0	0
	20–39	178	27	23	79	4	4	27	3	3	0	0	0
	40+	92	5	5	88	5	4	103	7	7	64	1	1
	Unknown	0	0	0	3	0	0	2	1	1	4	0	0
Full sibling	0–8	128	13	8	49	4	1	44	0	0	12	0	0
	9–19	120	10	5	52	3	2	53	4	2	12	1	1
	20–39	44	3	3	41	5	5	61	5	5	48	2	2
	40+	8	0	0	18	0	0	30	1	1	75	1	1
	Unknown	0	0	0	4	0	0	2	0	0	2	0	0
Maternal half sibling	0–8	13	1	1	15	1	1	6	2	2	0	0	0
	9–19	18	0	0	13	0	0	3	0	0	0	0	0
	20–39	3	0	0	3	0	0	5	0	0	0	0	0
	40+	0	0	0	4	0	0	4	0	0	2	0	0
	Unknown	0	0	0	0	0	0	1	0	0	1	0	0
Child	0–8	7	0	0	16	0	0	23	0	0	15	1	1
	9–19	5	1	1	5	0	0	10	1	1	13	0	0
	20–39	4	2	2	0	0	0	8	0	0	16	2	2
Total		622	62	48	390	22	17	382	24	22	264	8	8

APPENDIX B

Age Distribution of Relatives of Probands with Organic Major Motor Epilepsy, Focal Epilepsy, and Mixed Epilepsy

Relationship	Age	Organic Major Motor			Focal			Mixed		
		Number	+ all spells	+ maj. mot. spells	Number	+ all spells	+ maj. mot. spells	Number	+ all spells	+ maj. mot. spells
Parent	20–39	71	3	1	90	5	3	75	4	4
	40+	99	3	3	82	3	3	123	7	5
	Unknown	0	0	0	6	1	1	2	0	0
Full sibling	0–8	68	2	2	83	1	1	58	4	3
	9–19	76	1	0	97	3	2	89	3	3
	20–39	70	1	1	30	1	1	57	3	3
	40+	23	1	1	34	0	0	35	2	2
	Unknown	0	0	0	10	0	0	2	0	0
Maternal half sibling	0–8	9	0	0	11	0	0	4	0	0
	9–19	13	0	0	1	0	0	1	0	0
	20–39	13	0	0	2	0	0	1	0	0
	40+	0	0	0	3	0	0	0	0	0
Child	0–8	9	0	0	8	0	0	7	1	1
	9–19	7	0	0	5	0	0	3	0	0
	20–39	11	0	0	2	0	0	0	0	0
	40+	0	0	0	2	0	0	0	0	0
Total		469	11	8	466	14	11	457	24	21

APPENDIX C

Age Distribution of Relatives of Probands with Petit Mal, Minor Motor, and Psychomotor Epilepsy. Age Distribution of Relatives of Controls.

Relationship	Age	Petit Mal			Minor Motor			Psychomotor			Control		
		Number	+ all spells	+ maj. mot. spells	Number	+ all spells	+ maj. mot. spells	Number	+ all spells	+ maj. mot. spells	Number	+ all spells	+ maj. mot. spells
Parent	9–19	0	0	0	2	0	0	0	0	0	1	0	0
	20–39	40	0	0	49	2	1	7	0	0	460	16	12
	40+	28	1	0	11	0	0	15	0	0	468	9	7
	Unknown	0	0	0	0	0	0	0	0	0	11	0	0
Full sibling	0–8	31	1	1	25	0	0	12	0	0	484	18	12
	9–19	26	0	0	22	0	0	7	0	0	432	14	5
	20–39	2	0	0	4	1	0	6	0	0	359	12	7
	40+	0	0	0	0	0	0	11	0	0	236	2	2
	Unknown	0	0	0	0	0	0	0	0	0	28	0	0
Maternal half sibling	0–8	1	0	0	1	0	0	1	0	0	50	0	0
	9–19	1	0	0	3	0	0	0	0	0	38	1	1
	20–39	0	0	0	3	0	0	0	0	0	39	2	1
	40+	0	0	0	0	0	0	0	0	0	17	0	0
	Unknown	0	0	0	0	0	0	0	0	0	4	0	0
Child	0–8	1	0	0	0	0	0	0	0	0	89	3	2
	9–19	0	0	0	0	0	0	0	0	0	58	1	0
	20–39	0	0	0	0	0	0	1	0	0	78	2	1
	40+	0	0	0	0	0	0	1	0	0	6	0	0
Total		130	2	1	120	3	1	61	0	0	2858	80	50

DISTRIBUTION OF Cr^{51}-LABELLED ENDOTOXIN IN CORTISONE-TREATED MICE

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Mice can be protected from the lethal action of endotoxins by the prior administrations of antibiotics (1), chlorpromazine (2) or cortisone (3) or by establishing tolerance through repeated injections of sublethal doses of endotoxin (4). While the mechanisms of protection by these agents are unknown, the resistance of tolerant mice is probably related to the increased hepatic and pulmonary removal of endotoxin from the blood (4). The following study was undertaken to learn whether protection by cortisone is also associated with a change in tissue localization and blood clearance of endotoxin labelled with radiochromium.

MATERIALS AND METHODS

The effect of cortisone on distribution of radioactive endotoxin was examined in both normal and tolerant mice. Sixteen mice from a group of 32 non-tolerant mice were given 5.0 mg. cortisone acetate (Merck) into the thigh muscles eight hours before the injection of .75 mg. Cr^{51}-labelled endotoxin into the tail vein. Another group of 32 mice was made tolerant by 8 injections of endotoxin (not radioactive) intraperitoneally, according to the following schedule: .025 mg. on day 1; .05 mg. on day 3; .10 mg. on each alternate day thereafter. Three days after the last injection, 16 of the tolerant mice were given 5.0 mg. cortisone acetate intramuscularly, and 8 hours later Cr^{51}-labelled endotoxin was injected intravenously for tracer studies. On the day of all tracer experiments, the LD_{50} was calculated as previously described after giving graded intraperitoneal injections of endotoxin to normal and tolerant animals which had received simultaneously the same doses of cortisone or endotoxin as the test animals (4).

The endotoxin was extracted with trichloracetic acid from *Escherichia coli* grown on synthetic media, and labelled with $Na_2Cr^{51}O_4$. The exact technique for the preparation of endotoxin and its labelling with $Na_2Cr^{51}O_4$ has already been described in detail (4) and (5).

Labelled endotoxin was suspended in saline and injected in volumes of less than 0.5 ml. into the tail veins of Swiss albino mice weighing 15 to 30 grams. At 3, 15, 60, and 120 minutes after the injection 4 animals in each group of 16 were anesthetized with ether and blood was drawn from the heart. After the blood was drawn, the mouse was killed by a blow on the head and the tissues were removed and weighed. An aliquot of the blood was diluted and assayed directly for radioactivity. To determine the total amount of endotoxin circulating in blood, the blood volume of the mouse was assumed to be 12.1 per cent of the body weight

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(6) instead of 5 per cent as previously estimated (4). The organs were soaked in tap water at 4°C. until the wash water was visibly free of hemoglobin and were then placed in test tubes and digested with concentrated nitric acid. The total organ dissolved in nitric acid was assayed for radioactivity with a gamma-sensitive sodium-iodide thallium scintillating well-type crystal. The water used for soaking the tissues of each mouse was pooled. The pooled washings from each mouse and the mouse carcasses remaining after removal of the organs were also assayed for radioactivity. Each determination of radioactivity in blood and tissues was compared directly with that of a standard prepared by measuring radioactive endotoxin in the same syringe used for the injections into the mice.

In studies of the intracellular distribution of endotoxin, the livers of control and cortisone treated mice were removed two hours after the intravenous injection of 0.25 ml. of labelled endotoxin containing 1.7 mg. of endotoxin. The livers were perfused with 10 to 20 ml. of 0.25 M sucrose and then broken up in a Dounce homogenizer with ten volumes of 0.25 M sucrose. After homogenization the mitochondria, microsomal particles and supernatant fluid were separated by differential centrifugation at 4°C., and the amount of radioactivity in each subcellular fraction was measured.

RESULTS

I. The effect of cortisone on the distribution of labelled E. coli endotoxin

The capacity of cortisone to protect mice from the lethal action of endotoxin was confirmed. The LD_{50} in the cortisone-treated group was 1.7 mg. and in the non-treated control group 0.4 mg. On the other hand, the distribution of

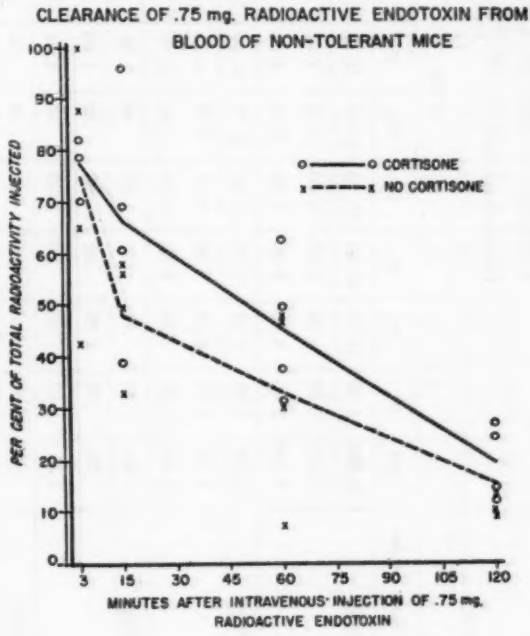

FIG. 1

TABLE I

Concentration of Radioactivity in Various Tissues of Control Mice

Concentration Index*— $\frac{\text{Per Cent Total Injected Radioactivity†}}{\text{Per Cent Total Body Weight‡}}$

Time	0 hr.				15 min.				1 hr.				2 hrs.			
Mouse Number	24	29	31	32	21	22	25	28	23	26	27	30	17	18	19	20
Blood	42.95†	8.35	13.51	47.89	17.16	24.47	22.32	10.60	12.18	8.02	.82	7.74	1.43	8.14	2.22	1.43
	5.91‡	2.38	2.50	5.45	4.20	5.13	4.82	4.00	3.11	3.26	1.46	2.00	1.75	3.50	2.74	1.50
	7.28*	3.50	5.40	8.80	4.09	4.76	4.64	2.65	3.92	2.46	.56	3.88	.82	2.32	.81	.95
Liver	6.33	7.08	5.59	5.70	25.06	18.52	21.30	25.06	25.67		23.22	12.63	32.85	29.83	36.32	41.06
	6.10	6.51	6.74	5.56	6.83	5.67	6.06	9.82	4.88		5.07	7.34	5.15	4.90	6.79	8.72
	1.04	1.08	.84	1.02	3.66	3.26	3.51	2.56	5.26		4.58	1.72	6.38	6.08	5.35	4.71
Lungs	.81	.55	.81	.74	.67	.49	1.07	.89	.85	.62	.43	.72	.90	.99	.71	.52
	.76	.75	1.23	.82	.82	.89	1.00	1.15	.90	.87	.85	.91	1.02	1.00	1.09	1.03
	1.07	.73	.66	.90	.82	.55	1.07	.77	.94	.71	.51	.79	.87	.99	.65	.50
Heart	.30	.36	.56	.35	.16	.19	.23	.16	.16	.14	.05	.27	.09	.18	.13	.11
	.56	.54	.72	.67	.46	.61	.46	.57	.52	.50	.49	.51	.47	.72	.65	.60
	.54	.67	.78	.52	.35	.31	.50	.28	.31	.28	.10	.52	.19	.25	.20	.18

Kidneys	1.40		1.34	1.10	.63	.77	.98	1.01	.89	.56	.43	1.16	.62	.89	.75	.63
	1.54		1.63	1.75	1.80	1.61	1.81	2.11	1.63	1.53	1.60	1.60	1.58	1.76	2.05	1.72
	.91		.82	.63	.35	.48	.54	.48	.55	.37	.27	.72	.39	.51	.37	.37
Spleen	.02	.06	.04	.04	.44	.24	.28	1.06	.57	.61	.52	.90	.79	1.20	.46	.39
	.35	.60	1.07	.29	1.05	.65	.60	2.02	.23	.52	.74	.59	.46	.62	.61	.46
	.06	.10	.04	.14	.42	.37	.47	.52	2.48	1.17	.70	1.53	1.71	1.94	.75	.85
Intestine	1.75	1.43	1.48	.99	1.29	1.24	1.51	2.16	3.57	2.04	1.43	2.27	1.59	2.65	2.21	1.48
	13.28	12.25	13.35	12.10	11.36	15.06	12.12	4.62	12.4	11.27	11.49	12.59	10.38	11.62	12.40	13.56
	.13	.12	.11	.08	.11	.08	.12	.47	.29	.18	.12	.18	.15	.23	.18	.11
Muscle	.29	.25	.37	.25	.26	.16	.38	.33	.31	.79	.20	.34	.54	.52	.50	.62
	2.59	1.96	2.08	2.68	2.73	2.52	3.44	2.79	3.24	4.88	2.28	2.90	3.04	4.04	4.71	5.50
	.11	.13	.18	.09	.10	.06	.11	.12	.10	.16	.09	.12	.18	.13	.11	.11
Bone	.05	.14	.11	.15	.37	.07	.18	.25	.18	.26	.06	.16	.15	.17	.41	.11
	.08	.18	.33	.51	.63	.45	.24	.40	.32	.32	.33	.21	.22	.28	.65	.18
	.63	.78	.33	.29	.59	.16	.75	.63	.56	.81	.18	.76	.68	.61	.63	.61
Brain	.22	.27	.54	.24	.14	.11	.13	.14	.20	.07	.04	.16	.09	.11	.08	.04
	1.82	1.98	1.71	1.90	1.44	2.05	1.69	1.99	1.77	1.77	1.73	1.89	2.00	1.90	1.52	1.62
	.12	.14	.32	.13	.10	.05	.08	.07	.11	.04	.02	.08	.04	.06	.05	.02

Each mouse received .75 mg. of endotoxin intravenously at zero time.

TABLE II

Concentration of Radioactivity in Various Tissues of Cortisone Treated Mice

Time	0 hr.				15 min.				1 hr.				2 hrs.			
Mouse Number	10	13	14	15	1	5	9	16	3	11	12	8	2	7	4	6
Blood	37.46	24.10		43.74	7.54	19.42	24.83	13.21	17.90	18.93	14.80	7.62	1.62	7.92	9.63	10.18
	5.79	4.19		6.47	2.38	3.41	3.16	2.66	6.85	4.65	4.74	3.57	1.42	6.31	4.80	4.44
	6.46	5.75		6.76	3.16	5.69	7.86	4.96	2.61	4.07	3.12	2.14	1.04	1.24	2.00	2.30
Liver	1.92	3.75	3.63	2.65	13.78	15.00	15.98	12.02	19.03	29.54	27.18	31.90	32.52	41.62	33.21	25.73
	7.69	7.90	6.28	7.05	8.03	7.74	9.26	8.05	7.26	7.80	7.87	6.96	8.85	7.11	7.93	7.31
	.25	.47	.58	.38	1.72	1.94	1.73	1.50	2.62	3.79	3.45	4.58	3.68	5.86	4.18	3.52
Lungs	1.32	.67	1.18	.85	.47	1.03	.92	.87	1.13	1.03	1.53	.99	.93	.61	.61	1.20
	.89	.76	.68	1.03	.92	1.17	.74	.76	.99	.89	.86	.97	.80	.85	.71	.92
	1.48	.88	1.74	.83	.51	.88	1.24	1.14	1.14	1.16	1.78	1.02	1.16	.72	.86	1.30
Heart	.61	.34	.41	.39	.09	.31	.48	.35	.13	.16	.20	.25	.11	.11	.19	.36
	.62	.60	.68	.68	.51	.56	.74	.61	.60	.48	.66	.63	.55	.59	.73	.75
	.98	.57	.60	.57	.18	.55	.65	.57	.22	.33	.30	.40	.20	.19	.26	.48

Kidney	1.06	.67	1.14	.94	.41	1.19	1.24	1.06	.77	1.20	.85	.87	.72	.70	.99	.86
	1.85	1.34	1.80	1.75	2.07	1.74	1.84	1.46	1.72	1.80	1.74	1.76	1.96	2.02	1.70	1.67
	.57	.50	.63	.54	.20	.68	.67	.73	.45	.67	.49	.49	.37	.35	.58	.51
Spleen	.02	.02	.05	.03	.30	.34	.36	.23	.84	.69	1.50	.99	.88	1.17	.57	1.06
	.43	.49	.27	.45	.62	.53	.66	.27	.50	.51	.55	.40	.79	.51	.30	.47
	.04	.04	.19	.07	.48	.64	.55	.85	1.68	1.35	2.73	2.48	1.11	2.29	1.90	2.26
Intestine	1.64	1.29	1.07	2.18	.81	1.36	1.57	1.19	1.49	1.84	1.86	1.51	1.91	1.38	1.28	2.11
	12.71	14.31	12.57	14.71	10.55	10.20	13.52	10.78	15.34	12.01	11.83	10.99	11.83	12.18	11.06	14.09
	.13	.09	.09	.15	.08	.13	.12	.11	.10	.15	.16	.14	.16	.11	.12	.15
Muscle	.64	.39	.20	.43	.68	.27	.31	.34	.38	.34	.51	.23	.36	.24	.31	.46
	4.71	2.65	3.21	4.78	4.75	3.77	4.97	4.41	5.72	4.38	5.73	2.47	3.27	3.21	3.41	4.26
	.14	.15	.06	.09	.14	.07	.05	.08	.07	.08	.09	.09	.11	.07	.09	.11
Bone	.14	.22	.20	.09		.3	.44	.44	.59	.22	.55	.62	.34	.69	.53	
	.67	.64	1.18	1.48		.61	1.06	.83	1.06	.53	1.08	.97	.81	1.56	.63	
	.21	.34	.17	.06		.49	.42	.53	.56	.42	.51	.64	.42	.44	.84	
Brain	.28	.26	.24	.34	.10	.19	.20	.30	.08	.13	.16	.12	.08	.05	.08	.09
	1.51	1.87	1.63	2.18	2.00	1.64	1.96	1.93	2.23	1.95	2.19	2.18	1.78	2.00	1.66	2.07
	.19	.14	.15	.16	.05	.12	.10	.16	.04	.07	.07	.06	.04	.03	.05	.04

Each mouse received .75 mg. of endotoxin intravenously at zero time.

labelled endotoxin in these two groups was similar. The clearance of 0.75 mg. of endotoxin from the blood is depicted in Figure 1. It is apparent that some of the endotoxin was removed from the blood, but at the end of one hour approximately 40 per cent of the injected endotoxin was still circulating. From the average values it appears that cortisone slightly impeded the removal of endotoxin; however, the differences are not significant.

Table I shows the amount and concentration of activity in the organs of control mice at varying times after the injection of labelled endotoxin, and Table II gives the same data for cortisone-treated mice. In both groups the lung, heart, kidney, spleen, intestine, muscle, and bone contained only small amounts of the injected radioactivity. Activity in the liver increased with time and reached 30 to 35 per cent at the end of two hours. These values were essentially the same in cortisone-treated and control mice.

A slower clearance of labelled endotoxin from the blood was observed after the injection of 1.5 mg. of endotoxin than after injection of 0.75 mg. (Fig. 2). Even at the end of two hours, 30 per cent of the endotoxin was still in the blood. The clearance of this larger dose was not influenced significantly by cortisone.

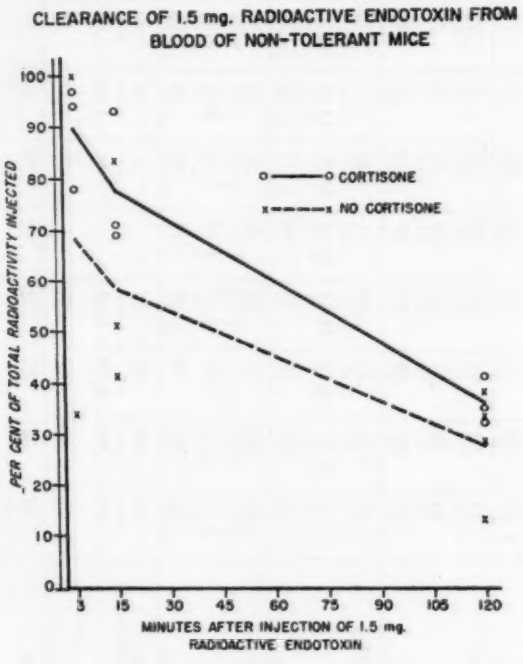

FIG. 2

These results indicate that the protection of mice by cortisone is not associated with a more rapid removal of endotoxin from the blood.

II. The effect of cortisone on the distribution of labelled endotoxin in tolerant mice

Tolerant mice were more resistant to the lethal action of endotoxin (LD_{50}—1.4 mg.) than non-tolerant control mice (LD_{50}—0.4 mg.). This resistance was not affected by administering cortisone eight hours before challenge; in a group of tolerant, cortisone-treated mice the LD_{50} was 1.6 mg. This resistance was accompanied by extremely rapid clearance of 1.5 mg. of labelled endotoxin as shown in Figure 3. At 15 minutes after injection the blood contained only 10 per cent of the injected activity.

In the present studies, tolerant animals did not exhibit greater removal of endotoxin by the liver and spleen than did control animals. The more rapid clearance from the blood was due in part to the early accumulation of endotoxin in the lungs (Fig. 4), a finding associated with high levels of precipitins against endotoxin (4). Neither the clearance of endotoxin nor its striking accumulation in the lung was affected by cortisone.

These experiments confirmed the findings of accelerated removal of endo-

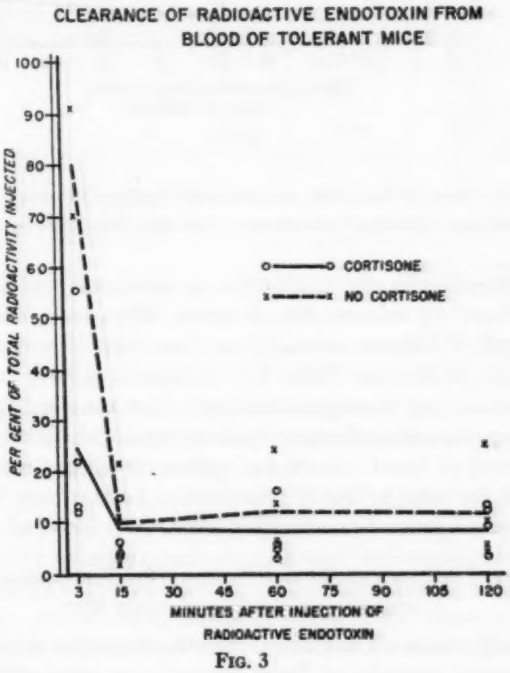

FIG. 3

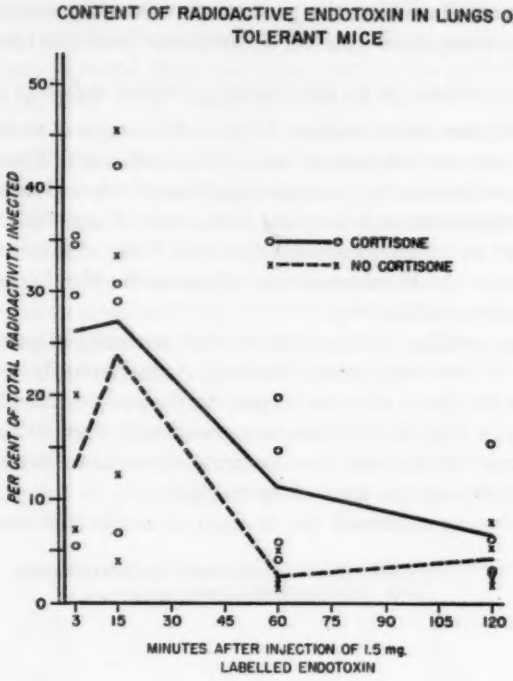

FIG. 4

toxin from the blood of tolerant animals and further demonstrated that cortisone did not reduce this rapid clearance or reverse the protection accompanying tolerance.

Another alteration in the distribution of endotoxin was noted in tolerant animals at 60 and 120 minutes after injection. More radioactivity was washed from the organs of tolerant animals than from non-tolerant animals and was recovered in the wash water (Table III). It seemed unlikely that the increased amount of elution from the organs of tolerant mice was due to greater retention of blood, inasmuch as the volume of retained blood (calculated as the difference between theoretical blood volume and volume of sample removed at cardiopuncture) was the same as that of control mice. Furthermore, blood of tolerant animals contained much less activity per ml. than blood of control animals.

III. The effect of cortisone on the distribution of labelled endotoxin in subcellular cytoplasmic particles of mouse liver

In preliminary studies it was found that concentration of labelled endotoxin in the microsomal particles of livers from mice treated with cortisone was

TABLE III

Per Cent of Injected Dose of 1.5 mg. of Labelled Endotoxin in Water Used for Soaking Organs

	3 min. after inj.	15	60	120
Tolerant mice	25.4	40.4	44.4	39.2
	22.9	32.4	37.8	40.8
		20.3	42.2	35.6
		41.2	39.8	29.3
Average	24.2	33.4	41.0	36.2
Tolerant animals (cortisone treated)	13.8	26.3	46.2	46.8
	22.5	16.9	44.3	29.8
	26.4	19.2	43.2	39.0
	34.3	19.7	16.7	37.9
Average	24.3	20.5	37.6	38.4
Normal animals	28.6	31.1		22.5
	25.0	17.6		28.8
Average	26.8	22.0		23.4
Normal animals (cortisone treated)	25.2	22.6	19.7	21.6
	18.4	22.8	24.8	11.7
	27.4	23.2	24.6	19.4
Average	23.7	22.9	23.0	20.9

TABLE IV

Per Cent of Cytoplasmic Endotoxin in Microsomal Particles of Mouse Liver

Control mice	18.0 ± 2.0%
Cortisone treated mice	14.3 ± 2.1%
	$P < 0.01$

slightly but significantly less than the concentration in microsomes of control animals (Table IV). The concentrations in mitochondria and supernate were not significantly different in the two groups.

DISCUSSION

These experiments establish that the increased resistance to endotoxin in cortisone-treated mice is not due to a more rapid clearance of circulating endotoxin. In this way, protection by cortisone differs from tolerance.

It is interesting to compare these experiments with those of Chedid in parabiotic mice (8). One of the parabiotic pair received 1.5 lethal doses of endotoxin

plus cortisone. The partner received 0.2 lethal doses of endotoxin and no cortisone. The mouse that had received the smaller dose of endotoxin and no cortisone succumbed, and the cortisone-treated partner survived in 7 cases out of 10. Because cortisone did not cross the parabiotic bridge, the authors concluded that cortisone protected the animal into which it was injected while the unaffected endotoxin was free to pass into the untreated animal and kill it. They also found that cortisone did not inactivate endotoxin *in vitro*. These experiments in parabiotic mice thus support our finding that cortisone does not accelerate the removal of endotoxin from the blood of cortisone-treated mice.

If cortisone does not increase clearance of endotoxin from the blood or alter its distribution in organs, by what mechanism does it protect? It has been shown that the protective action of the steroids is not antihistaminic in nature (9) and that the mechanism of protection is different from that observed with chlorpromazine (10). Cortisone is protective in the complete absence of the thymus, spleen, and partial absence of the liver (11). It is conceivable that cortisone alters the distribution of endotoxin in a manner too subtle to detect by assay of endotoxin in whole organs. The possibility must be considered, therefore, that access of endotoxin to a susceptible target inside the cell is blocked because of decreased permeability of the cell membrane or altered intracellular distribution of endotoxin. In preliminary studies, we have found that distribution of labelled endotoxin in subcellular particles of liver cells may be altered slightly by cortisone (Table IV).

The finding that cortisone does not diminish or augment the resistance of tolerant mice has previously been reported in adrenalectomized, tolerant mice (12). In rabbits, cortisone does not influence pyrogen tolerance, but abolishes the protection from the localized Shwartzman reaction seen in tolerant animals (13).

The increased elution of radioactivity from the tissues of tolerant mice can be correlated with the observation that endotoxin leaves the lung of tolerant animals approximately one hour after heavy initial localization (Fig. 4), (4). It has been postulated that endotoxin combines instantly with precipitins in tolerant mice and that the precipitates are filtered out by the pulmonary capillaries (4). Because it is unlikely that phagocyted particles could leave the pulmonary macrophages and reappear so rapidly in the blood, it is believed the endotoxin-antibody precipitates are simply trapped in the capillaries of the lung, possibly in circulating neutrophiles, and are later washed out in the general circulation.

SUMMARY

(1) Doses of cortisone that prevented death did not materially influence the rate of removal or organ distribution of lethal amounts of radioactive *E. coli* endotoxin in normal mice.

(2) Cortisone neither enhanced nor reduced the resistance of tolerant mice to *E. coli* endotoxin or their ability to clear radioactive endotoxin from the blood into the lung and other organs.

(3) It is concluded that protection by cortisone, unlike that of tolerance, is independent of the blood and whole tissue concentration of endotoxin.

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STUDIES ON THE RENAL EXCRETION OF SALICYLATE IN THE DOG[1]

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Salicylate is eliminated from the body almost exclusively by the renal excretion of the drug and its metabolites (1, 2, 3, 4). Recent studies have shown that the excretion of salicylate, but not its metabolites, is markedly influenced by the pH of the urine (2, 5, 6). The present studies are concerned only with unchanged salicylate. The output of salicylate is much higher when the urine is alkaline than when acid (2, 5, 6, 7, 8, 9, 10). Under circumstances with an acid or neutral urine, the salicylate clearance suggests the tubular reabsorption of the major portion of that which is filtered. Conversely, when the urine is extremely alkaline, the amount of salicylate excreted may exceed the amount filtered at the glomerulus. This indicates net movement of salicylate from extracellular fluid to tubular urine. The nature of this process has been interpreted by Berliner (11) and by Macpherson *et al.* (5) as the passive diffusion of the free acid into the more alkaline fluid within the lumen of the tubule. This has been referred to as non-ionic diffusion or ion trapping (see review by Milne *et al.* (12)). However, Gutman *et al.* (7) and Schachter and Manis (6) have shown that salicylate secretion can be depressed by an inhibitor of active transport, probenecid.

In an attempt to clarify the renal mechanisms of salicylate excretion, experiments have been carried out with the clearance technique in the dog. The relative roles of urinary pH, urine volume, and active transport have been assessed.

METHODS

Experiments were carried out on trained, unanesthetized, female, mongrel dogs which had been fasted and deprived of water for 18 hours. Standard methods for renal clearances were employed. In most experiments the dogs were primed with 0.3 M sodium acetate, 20 to 25 ml./kg.; a sustaining infusion of 0.3 M sodium acetate was continued throughout the experiment at a rate which was approximately 10 per cent of the filtration rate. The initial dose of salicylate was approximately 33 mg./kg. Suitable amounts of creatinine and sodium salicylate were added to the infusions to maintain the plasma levels at about 20 mg. per cent for creatinine and 8 to 15 mg. per cent for salicylate. Urine samples were taken through an

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indwelling catheter; the bladder was washed at the end of each collection period with 10 ml. of distilled water. Blood samples were taken from the external jugular vein at the midpoint of each ten minute clearance period. In the middle of each period a small sample of urine was collected from the bladder under oil and the pH determined immediately. For pH determination jugular vein blood was handled anaerobically. The standard experiment involved a thirty minute equilibration period after the loading infusion, three ten minute control periods, a discard period following the injection of the test substance, followed by three ten minute experimental periods. Deviations from this protocol are described in the tables.

pH was determined with a Beckman model G meter fitted with an anaerobic electrode. The observed pH value was corrected to 37°C. using a factor of 0.01 units per degree. Creatinine was determined by the alkaline picrate method on trichloracetic acid filtrates of plasma, and on appropriately diluted urine samples to which an equivalent amount of trichloroacetic acid had been added. Salicylate was measured by the ethylene dichloride extraction procedure of Brodie *et al.* (13). Since salicylurate is partially extracted by this procedure and contributes to the final chromogenicity, duplicate sets of analyses were made using carbon tetrachloride extraction. Since different proportions of salicylurate are extracted by these two solvents the presence of significant quantities of salicylurate would be revealed by differences in the apparent concentration of salicylate in the samples (13, 2). In no case did the difference between the two methods exceed the analytical errors, and it is concluded that salicylurate excretion was neglibible under the conditions of these experiments. Other metabolites of salicylate are not measured by this procedure (13).

Ultrafiltrates of plasma were prepared from four samples from each experiment by the method of Toribara *et al.* (14) at 37°C. in an atmosphere of 5 per cent carbon dioxide in oxygen. The clearance of salicylate has been calculated as UV/F in which F is the concentration in plasma ultrafiltrate.

p-Aminohippurate (PAH) was determined by the Bratton and Marshall method (15). Tubular transport of PAH was calculated without consideration for plasma protein binding. Acetazolamide was determined in plasma ultrafiltrates and in urine by a polarographic method, the details of which will be reported in a subsequent paper. The recoveries from plasma and urine with this method are 95 ± 2 per cent.

RESULTS

In the early experiments, infusions of 0.15 N sodium bicarbonate were employed, but ratios of salicylate clearance to filtration rate (C_{sal}/GFR) greater than unity were obtained only occasionally. Sodium acetate was substituted for bicarbonate because of the stimulatory effect of this salt on a presumably related transport mechanism, the tubular secretion of *p*-aminohippurate (PAH) (16). In addition, the infusions were made hypertonic (0.3M) in order to obtain a maximal bicarbonate concentration (and thus pH) in the urine. With this procedure, C_{sal}/GFR ratios greater than unity have been consistently observed. Comparison of our results with those obtained by others in man (6, 7) and in the rabbit (8) leads to the impression that the dog may secrete salicylate less readily than the other species studied. Smith (17) has noted a similar deficit of PAH transport in the dog. In retrospect, our data do not definitely establish a stimulatory effect of acetate on salicylate transport

TABLE I

Effect of Acetazolamide on Salicylate Clearance With Initially Acid Urine

Time	V	GFR	Urine	Salicylate			C_{sal}/GFR
				F	UV	UV/F	
mins.	*ml/min.*		*pH*	*mg%*	*mg./min.*	*ml./min.*	
0–10	0.4	25	6.55	4.0	0	0	0
10–20	0.5	34	6.72	3.4	0	0	0
20–30	0.4	32	6.70	3.4	0	0	0
30–35	Acetazolamide 15 mg./kg. i.v.						
45–55	0.6	18	7.93	3.0	.094	2.8	0.16
55–65	1.4	31	8.00	3.0	.177	5.8	0.19
65–75	1.1	23	7.92	3.6	.131	4.4	0.19

Dog M, 15.2 kg. Sustaining infusion of 0.15 N NaCl throughout experiment at 2 ml./min. containing sodium salicylate and creatinine. F = 0.40 plasma salicylate.

Abbreviations in this and subsequent tables: V, urine volume; GFR glomerular filtration rate; F, concentration in plasma ultrafiltrate; UV, urinary concentration x urine volume (V); UV/F, clearance calculated on basis of ultrafiltrable salicylate; C_{sal}/GFR, ratio of clearance of ultrafiltrable salicylate to GFR.

because of the concomitant rise in urinary pH due to the hypertonicity of the infusions. Unequivocal salicylate secretion may be observed in the absence of acetate, cf. Table V.

Thirty-six experiments were performed. Because of the number of variables which were examined, the data will be presented by individual protocols. The dose of salicylate resulted in plasma concentrations between 8 and 15 mg. per cent in all experiments. The concentration of salicylate in plasma ultrafiltrates ranged from 30 to 53 per cent of the plasma concentration. This value was constant for each individual experiment and was not affected by the compounds used to modify salicylate excretion.[3]

Effect of Acetazolamide. In confirmation of the findings of others (5), the administration of acetazolamide increases the excretion of salicylate when the urine is initially acid (Table I), an effect which can be attributed to the increase in both urinary pH and volume. Under these circumstances there is a rise in the urinary concentration of salicylate.

On the other hand, when the urine is initially alkaline, acetazolamide decreases the excretion of salicylate (Table II, Fig. 1). In five experiments urine pH remained essentially unchanged and urine volume regularly increased. As will be discussed below, an increase in urine flow *per se* augments salicylate excretion. Hence, there is a tendency for the acetazolamide diuresis to mask

[3] For each experiment, the average value for the per cent of plasma salicylate that was ultrafiltrable was used for calculating salicylate clearance. The mean value from all experiments was 41 per cent. In any individual experiment this value showed no systematic variations. Within 95 per cent confidence limits, the maximal error attributable to measurement of protein binding is 6.4 per cent of the reported value.

TABLE II

Effect of Acetazolamide on Salicylate Clearance With Initially Alkaline Urine

Time	V	GFR	Urine	Salicylate			C_{sal}/GFR
				F	UV	UV/F	
mins.	*ml./min.*		*pH*	*mg.%*	*mg./min.*	*ml./min.*	
0–10	2.8	60	7.96	5.7	2.92	51	0.85
10–20	2.3	47	7.93	6.3	2.86	45	0.96
20–30	2.9	56	7.94	6.8	3.51	52	0.93
30–35	Acetazolamide 20 mg./kg. i.v.						
45–55	4.8	42	7.89	7.6	2.05	26	0.62
55–65	8.5	61	7.87	8.8	3.65	41	0.67
65–75	8.1	58	7.84	8.9	3.55	40	0.69

Dog J1, 18.5 kg. Sustaining infusion of 0.3 N sodium acetate throughout experiment at 6 ml./min. containing sodium salicylate and creatinine. F = 0.46 plasma salicylate.

the inhibitory effect of the drug on salicylate excretion. In spite of this, a significant depression of C_{sal}/GFR was consistently observed.

To determine whether this effect of acetazolamide depends upon carbonic anhydrase inhibition, three experiments (with a protocol similar to Table II) were performed with the t-butyl acetazolamide analogue.[4] Maren (18) has shown that this compound (Cl 13,850) is inactive as a carbonic anhydrase inhibitor, and that its conversion to an active compound in the dog is insignificant. Following the administration of 25 mg./kg. in two experiments and 35 mg./kg. in a third, there was no change in urine pH or volume. The average values for C_{sal}/GFR were: control, 1.44; experimental: 1.06. These results indicate that both acetazolamide and the analogue depress salicylate excretion to about the same extent. Hence, the effect does not depend on inhibition of carbonic anhydrase.

Competition for tubular transport is well established as a mechanism underlying the depression of the excretion of one compound by another (cf. (19) for review). Three experiments were therefore performed to determine whether or not acetazolamide is secreted by the renal tubule and whether this secretion could be inhibited by an agent which has a similar effect on salicylate secretion. As shown in Table III, under the conditions of these experiments, the clearance of acetazolamide is greater than the filtration rate and is markedly depressed by probenecid.[5] The depression of salicylate secretion by acetazolamide may be attributed to competition for the transport mechanism.

[4] Acetazolamide and 2-acetylamino-1,3,4-thiadiazole-5-sulfon-t-butyl amide (Cl-13,850) were generously supplied by the Lederle Laboratories Division of the American Cyanamide Co.

[5] Striking similarities exist between the renal mechanisms of salicylate and acetazolamide excretion. As shown here, both may be secreted by the tubule. Maren (20) has reported a pH dependence for acetazolamide excretion. A volume effect may be inferred by comparing the data of Maren *et al.* (21) with the present studies.

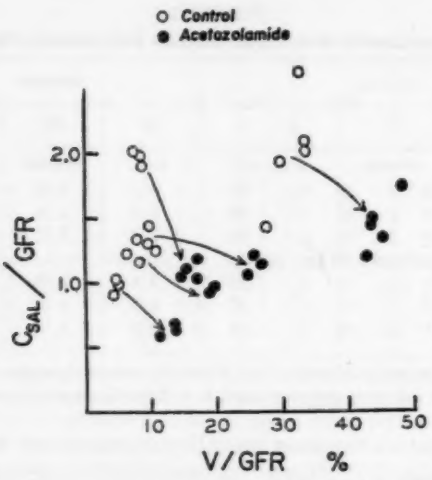


FIG. 1. Effect of acetazolamide on salicylate clearance with persistently alkaline urine. Control and experimental periods from each of five experiments are connected by arrows. Experiment with highest value for V/GFR was performed during diuresis produced by sodium acetate and mannitol, urine pH 7.43 to 7.59. Other experiments were similar to that of Table II; urine pH 7.80 to 8.07. The dose of acetazolamide was 15 mg./kg. in three experiments and 20 in the other two.

Effect of p-aminohippurate on salicylate secretion. Further characterization of salicylate transport is provided by the observation (see Table IV) that PAH inhibits salicylate excretion, urine pH and volume remaining constant. This is interpreted as a competitive phenomenon. Dalgaard-Mikkelson (8) has shown a marked depression of salicylate excretion in rabbits following the administration of *p*-aminobenzoid (PABA). Since PAH is a metabolite of PABA, it is not certain whether his results are attributable to PABA, PAH or other metabolites.

Effect of Probenecid and Benzmalecene.[6] Probenecid inhibits the tubular transport of various organic anions (19). Studies in man have shown that it depresses the excretion of salicylate (6, 7). This finding has been confirmed in the dog (cf. Table VI). Benzmalecene is a recently developed agent which has an effect on organic acid transport similar to that of probenecid (22). At a dose of 20 mg./kg. it depressed C_{sal}/GFR by an average of 40 per cent in experiments similar to those of Tables II and IV.

Effect of Urine Volume. Since it has been shown that the rate of urine flow may modify salicylate excretion (5), this factor was reevaluated under the

[6] Probenecid and Benzmalecene [N-(1-methyl-2,3-di-chlorophenylpropyl)-maleamic acid (α-isomer)] were generously supplied by the Merck Sharp and Dohme Research Laboratories.

TABLE III

Effect of Probenecid on Urinary Excretion of Acetazolamide

Time	V	GFR	Urine	Acetazolamide			$C_{acetaz.}/GFR$
				F	UV	UV/F	
mins.	*ml./min.*		*pH*	*mg.%*	*mg./min.*	*ml./min.*	
0–10	7.6	51	7.91	2.2	1.30	59	1.16
10–20	6.5	49	7.89	(1.9)	1.26	66	1.35
20–30	6.4	52	7.91	1.6	1.27	80	1.54
30–35	Probenecid 20 mg./kg. i.v.						
45–55	6.1	55	7.97	2.4	.42	18	0.33
55–65	8.4	53	7.93	(2.3)	.65	28	0.53
65–75	8.2	51	7.92	2.3	.66	29	0.57

Dog D, 15 kg. Sustaining infusion of 0.3 N sodium acetate at 5.6 ml./min. during control periods; rate increased to 8.2 ml./min. at time probenecid administered. Prior to observations dog received 15 mg./kg. i.v. Acetazolamide; this was also added to sustaining infusions so that rate of administration was 1.4 mg./min. Values in parentheses were obtained by interpolation.

TABLE IV

Effect of PAH on Excretion of Salicylate

Time	V	GFR	Urine	PAH		Salicylate			C_{sal}/GFR
				P	T	F	UV	UV/F	
mins.	*ml./min.*		*pH*	*mg.%*	*mg./min.*	*mg.%*	*mg./min*	*ml./min.*	
0–10	8.5	61	7.81			3.5	3.29	94	1.55
10–20	8.3	63	7.83			3.6	3.36	93	1.49
20–30	8.0	59	7.84			3.7	3.53	95	1.60
30–35	PAH 3 gms. i.v.								
35	PAH added to infusion to give 30 mg./min.								
45–55	7.6	55	7.85	45	17	4.1	1.84	45	0.82
55–65	8.3	62	7.87	44	19	4.0	2.10	53	0.85
65–75	7.9	56	7.90	44	19	4.4	2.00	45	0.81

Dog D, 15 kg. Sustaining infusion of 0.3 N sodium acetate maintained throughout experiment at 5.6 ml./min. F = 0.38 plasma salicylate. T = tubular transport.

conditions of these experiments. The results depicted in Figure 2 were obtained at an essentially constant alkaline urinary pH while urine volume progressively increased from 2.5 to 12 per cent of the filtration rate. The ratio of C_{sal}/GFR rose in a curvilinear fashion to a maximum at a V/GFR value of approximately 9 per cent. All experiments of the type shown in Tables III and IV were performed at high rates of urine flow, thus avoiding the marked effects of variations in volume at low rates of flow.

In order to elucidate further the relative effects of urine volume and pH, seven experiments were performed at the very high rates of urine flow produced by mannitol diuresis. Protocols are given in Tables V and VI. By critical

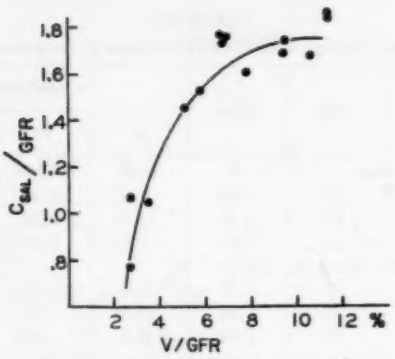


FIG. 2. Effect of urine volume on salicylate clearance with persistently alkaline urine. Dog D, 15 kg. At −35 mins. dog received priming infusion 150 ml. of 0.3 N sodium acetate with salicylate and creatinine. Sustaining infusion of 0.3 N sodium acetate at 1.7 ml./min. was maintained until zero time when rate was increased to 9.0 ml./min. Amounts of sodium salicylate and creatinine in infusion were adjusted to maintain constant plasma levels (9 to 11 mg./100 ml. for salicylate). Urine pH varied from 8.10 at low urine flows to 7.88 at highest urine flow. GFR increased from 57 to 69 ml./min. during period of observation.

TABLE V

Effect of Osmotic Diuresis on Salicylate Excretion

Time	V	GFR	Urine	Blood	Salicylate			C_{sal}/GFR
					F	UV	UV/F	
mins.	*ml./min.*		*pH*	*pH*	*mg.%*	*mg./min.*	*ml./min.*	
35	Priming infusion of 175 ml. of 0.15 N $NaHCO_3$ with creatinine and sodium salicylate.							
30	Sustaining infusion of 0.15 N $NaHCO_3$ at 1.7 ml./min.							
0–10	2.3	46	7.58	7.55	6.0	1.62	27	0.59
10–20	1.6	50	7.63	7.55	6.0	1.85	31	0.62
20–30	1.0	42	7.78	7.53	6.1	1.52	25	0.60
30–35	Injection of 150 ml. of 0.2 N $NaHCO_3$ containing 30 gms. mannitol.							
35	Start sustaining infusion 4% mannitol in 0.03 N $NaHCO_3$ at 25.5 ml./min.							
50–60	19.1	58	7.34	7.54	6.7	5.10	76	1.31
60–70	23.3	65	7.35	7.55	6.5	5.90	91	1.40
70–80	20.6	60	7.40	7.56	6.7	5.36	80	1.33

Dog D, 15.5 kg. Sustaining infusions contained appropriate amounts of creatinine and sodium salicylate (see Methods). F = 0.40 plasma salicylate. Blood samples were obtained from the jugular vein.

adjustment of the amounts of mannitol and sodium bicarbonate that were infused, it was possible in four experiments to demonstrate C_{sal}/GFR ratios significantly greater than unity when the pH of the urine was more acid than that of the blood. It may be noted that blood samples were obtained from the jugular vein. Therefore, the reported values for blood pH are lower than those

TABLE VI

Effect of Probenecid on Salicylate Excretion During Osmotic Diuresis

Time	V	GFR	Urine	Blood	Salicylate			C_{sal}/GFR
					F	UV	UV/F	
min.	*ml./min.*		*pH*	*pH*	*mg.%*	*mg./min.*	*ml./min.*	
46	Priming infusion of 250 ml. of 0.36 N $NaHCO_3$ containing 37.5 gms. mannitol with creatinine and sodium salicylate.							
38	Sustaining infusion of 5% mannitol in 0.075 N $NaHCO_3$ at 26 ml./min.							
0–10	31.7	76	7.45	7.55	3.7	4.15	112	1.47
10–20	33.1	79	7.46	7.55	4.2	4.17	99	1.26
20–30	33.2	84	7.46	7.56	3.8	4.71	124	1.48
30–35	Probenecid 20 mg./kg. i.v. and added to infusion at 37 mg./kg./hr.							
35–45	34.2	82	7.50	7.57	4.0	2.94	73	0.89
45–55	30.5	75	7.51	7.58	4.2	2.93	70	0.93
55–65	30.0	76	7.51	7.56	4.5	2.94	65	0.86

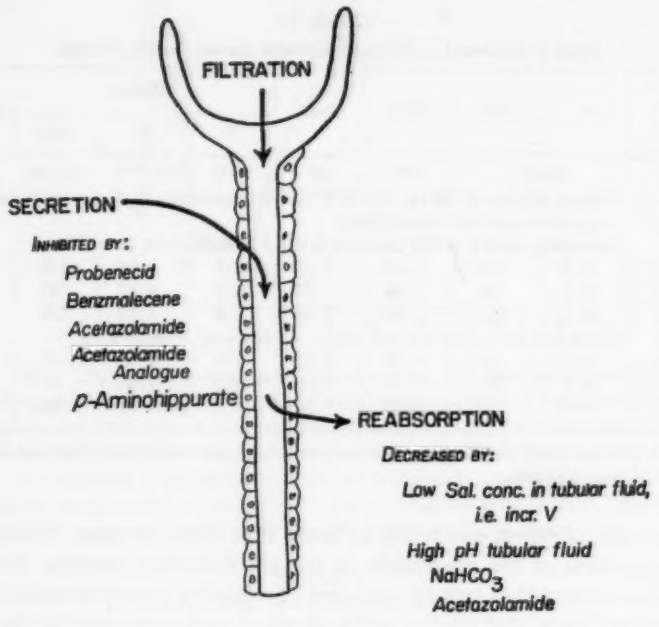
Dog T, 16.0 kg. Sustaining infusions contained creatinine and sodium salicylate (see Methods). F = 0.40 plasma salicylate.

for renal arterial blood and probably lower than those for renal venous blood (23). Regardless of the magnitude of the pH difference between blood and urine, it is evident that tubular secretion of salicylate cannot be attributed to ion-trapping, since the relative pH's of blood and urine are in the wrong direction for this to be operative. Under these conditions, the apparent secretion of salicylate is inhibited by probenecid (Table VI). The magnitude of this effect is approximately the same as that obtained at lower rates of urine flow.

Site of salicylate secretion. Several experiments were performed by the stop flow technique of Malvin, Wilde and Sullivan (24). When the urine was acid, an increased rate of salicylate reabsorption was noted in the more "distal samples". With an alkaline urine resulting from the infusion of sodium acetate, the values for the salicylate U/F ratio divided by the creatinine U/P ratio were: free flow, 2.0; "distal sample", 1.3; "best proximal sample", 2.5. Following the administration of acetazolamide, these values were, respectively: 1.25; 0.85; and 1.35. These findings are consistent with the proximal secretion and distal reabsorption of salicylate. However, the possibility is not excluded that both secretion and reabsorption may occur in any segment, but that their relative magnitudes may vary from one segment to another. Since PAH and salicylate are handled by a common mechanism and since PAH is secreted proximally, it is reasonable to identify salicylate secretion as a proximal process.

DISCUSSION

The factors influencing the secretion and reabsorption of salicylate are depicted in Figure 3 in accordance with the interpretation to be presented below.



URINARY EXCRETION OF FREE SALICYLATE

FIG. 3. Scheme of renal mechanisms regulating salicylate excretion.

These experiments demonstrate that salicylate is secreted[7] by active tubular transport. This conclusion is supported by several lines of evidence. First, the amount of salicylate excreted can be in excess of the quantity filtered. Secondly, the apparent secretory contribution is depressed by substances which are themselves secreted (i.e. PAH and acetazolamide), and also by other compounds (i.e. probenecid and benzmalecene) which inhibit the tubular secretion of various organic acids, including PAH and acetazolamide. The effects of these agents were noted while urinary pH and filtration rate remained constant. In those experiments in which there was a change in urine flow, this was in a

[7] A consideration of salicylate excretion serves to emphasize some semantic problems in renal physiology. Secretion will be used to denote the movement of a substance from the peri-tubular space to the tubular lumen. *Net* secretion indicates that, as a consequence of the secretory process, more of a substance is excreted in the voided urine than can be accounted for by the amount in the glomerular filtrate. Since the evidence strongly suggests that salicylate may simultaneously be both secreted and reabsorbed at either the same or different sites along the nephron, it is logical to consider the secretory process as operative under many conditions, including these in which *net* secretion may not be demonstrable.

direction tending to increase, rather than decrease, salicylate excretion. The above relationships indicate that these several organic anions are transported by a common mechanism. On the basis of this evidence there is no *a priori* reason for believing that the inhibition of salicylate excretion by either acetazolamide, probenecid or PAH is the result of increased salicylate reabsorption.

A different concept of the nature of the secretory process has been previously proposed by others (5, 11). This is the ion-trapping mechanism, according to which salicylic acid diffuses into the urine from the peritubular fluid along the concentration gradient for the free acid. This gradient has been presumed to increase with the rise in urinary pH. In the case of salicylate this explanation appears untenable because, first, net salicylate secretion can occur when the urine is more acid than the blood, and second, salicylate excretion is inhibited by agents (see above) which act on other active secretory mechanisms and which do not change urine pH.

Further considerations of ion-trapping as the mechanism underlying salicylate *secretion* reveal some unexpected implications when the factors of pH, urine flow, and filtration rate are all taken into account. Consider, for example, one of our experiments in which the following values were obtained during the infusion of sodium acetate: C_{sal} (calculated on the basis of plasma filtrate), 93 ml./min.; GFR, 60 ml./min.; V, 8.3 ml./min.; C_{sal}/GFR, 1.55; urine and blood pH, 7.83 and 7.63, respectively; observed U/F ratio for salicylate, 11.2. Values of this magnitude are frequently encountered in the dog when metabolic alkalosis is produced by infusions. The salicylate U/F ratio at equilibrium is given by the equation (cf. 25):

$$\frac{C_U}{C_P} = \frac{1/10^{(pH_U - pKa)}}{1/10^{(pH_P - pKa)}}$$

in which C represents concentration of total salicylate (ionized plus unionized), and the pKa for salicylic acid is 3.0. By definition, non-ionic diffusion is considered to reach an equilibrium when the concentration of free salicylic acid is the same on both sides of the lipid membrane. With the values given, the equilibrium U/F ratio for total salicylate would be 1.58, compared to the observed value of 11.2. To account for this type of discrepancy Macpherson *et al.* (5) have advanced the hypothesis that equilibrium across the tubule occurs at a proximal site and that a more distal segment reabsorbs water but is impermeable to salicylic acid, either ionized or unionized. This would increase the U/F ratio and thus permit better agreement between observation and theory. In order to evaluate this hypothesis further one assumption must be made which seems completely reasonable, namely that the pH gradient between plasma and tubular fluid at any point in the nephron is no greater than

the final gradient between plasma and voided urine.[8] For simplification, the difference between the pH of plasma and filtrate is considered negligible. On this basis one may calculate the volume of the hypothetical proximal fluid which would have to equilibrate with plasma. Assuming that the pH of this fluid is 7.83 (i.e. the same as the voided urine), its volume would be $11.2/1.58 \times V$. Since V is 8.3 ml./min., this would give a "proximal volume" of 7.1×8.3, or 58.7 ml./min., a value which is 98 per cent of the observed glomerular filtration rate.

Based on these calculations, the mechanism postulated by Macpherson *et al.* (5) appears untenable. It implies that maximal alkalinity is achieved when as little as 2 per cent of the filtrate has been reabsorbed. At this pH the bicarbonate concentration in the tubular fluid is approximately 60 per cent greater than in plasma, i.e. U/F bicarbonate ratio of ca. 1.6. It is evident that the reabsorption of 2 per cent of the filtered water could not concentrate this amount of bicarbonate within the tubular fluid. This, in turn, would necessarily imply that the urine is alkalinized by the tubular secretion of bicarbonate, carbonate or hydroxyl ions. The above analysis is based on the thesis that no salicylate is reabsorbed distal to the hypothetical proximal site. Therefore, the calculations further imply that there is complete impermeability to salicylate for almost the entire tubule, a conclusion of doubtful validity. Furthermore, it may be re-emphasized that the results obtained with mannitol diuresis can not be attributed to a secretory mechanism based on the ion-trapping hypothesis. From many points of view, therefore, this mechanism seems improbable.[9]

Although non-ionic diffusion fails to explain the tubular *secretion* of salicylate, many factors indicate that diffusion of this type is a major determinant of salicylate *reabsorption*. This view is consistent with the process of active secretion as well as with the marked dependence of overall *excretion* on urinary pH and urine flow. According to the concept of non-ionic diffusion (see (28) for review), the tubule may be considered impermeable to the salicylate ion but

[8] Recent studies on the urinary concentrating mechanism have indicated that during antidiuresis there is a progressive rise in the total solute concentrations in the blood, interstitial water, and tubular fluid as the papilla is approached from the cortex (cf. 26 for review). Since hypertonic infusions were employed in these experiments, it may be presumed that the kidney was under the influence of anti-diuretic hormone. The possibility arises that the concentrations of salicylate, bicarbonate, and carbon dioxide in the various fluids within the kidney paralleled the changing concentration of total solute. However on the basis of current concepts, the pH gradient between plasma and adjacent tubular fluid would remain constant despite changes in total osmolality.

[9] While the ion-trapping mechanism appears to be invalid as the basis for the secretion of salicylate (and probably other organic acids), the present analysis may not necessarily apply to the excretion of bases, which are "trapped" in *acid* urines (27). The hydrogen ion gradient between plasma and urine may become extremely large with an acid urine, but is always relatively small with an alkaline urine.

permeable to the free acid. Therefore, the rate at which salicylate diffuses out of the tubule (i.e. reabsorption) would depend on the concentration gradient of the free acid between tubular fluid and interstitial (or plasma) water. Three factors tend to make the concentration of the free acid in the tubular fluid greater than in plasma. These are: first, the active secretory transport of salicylate from renal plasma into the lumen of the tubule (presumably proximal); second, the reabsorption of water; and third, the acidification of the tubular fluid. For present purposes it may tentatively be assumed that the rate of active secretion is relatively constant.[10] Therefore, the two major variables which determine salicylate reabsorption are the pH and volume of the tubular fluid. For example, either a high urinary pH or a high rate of urine flow would lower the concentration of salicylic acid within the tubular fluid. This would decrease the concentration gradient from lumen to plasma, which, in turn, would decrease the rate of reabsorption and hence increase excretion. According to this concept, the direction of the *passive* diffusion of salicylic acid is always *out* of the tubule. In addition, intrinsic properties of the tubular membrane appear to impede the rate of back diffusion so that an equilibrium state is not achieved. In all experiments, the observed U/F ratios were higher than the equilibrium values calculated from the equation given above. If however, the equilibrium for salicylic acid were ever to be obtained, it would follow that the salicylate clearance would never be greater than the rate of urine flow, except when the urine was more alkaline than the plasma; in addition, even at maximal alkalinity of the urine, the salicylate clearance would only be fourfold greater than urine flow (from the equation above, the U/F ratio for salicylate is 4 at blood and urine pH's of 7.4 and 8.0, respectively).

The "paradoxical" effects of acetazolamide are readily explained by two completely independent actions—namely, competition for tubular transport and alkalinization of the urine. The latter, but not the former, involves carbonic anhydrase inhibition. It is of interest that acetazolamide has been reported to be significantly less effective than sodium bicarbonate in increasing the excretion of other organic acids. These include citrate (30), phenobarbital (31), the sulfonamides and gentisate (12). Studies are now in progress to ascertain to what extent these, and other acids, are excreted by mechanisms similar to those for salicylate.

SUMMARY

The renal excretion of salicylate has been examined in the dog. Since the amount excreted may be either greater or less than the amount filtered, tubular

[10] The true magnitude of secretion has as yet not been determined, since reabsorption by back diffusion may occur to a varying extent depending upon the factors cited. However, the assumption of constant secretion appears reasonable by analogy to PAH, the excretion of which, at low plasma levels, is independent of acid-base balance (29).

secretion and reabsorption must both be operative. Salicylate is secreted by an active transport mechanism resembling that of PAH. Salicylate and PAH are competitively secreted and both are depressed by probenecid. Acetazolamide inhibits salicylate secretion, an action attributable to competition for active tubular transport. The reabsorption of salicylate is considered to occur by passive diffusion of the unionized free acid. The dependence of salicylate excretion on urine pH and flow is interpreted in terms of the effect of these factors on the reabsorptive process.

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ION ASSOCIATION. II. THE EFFECT OF MULTIVALENT IONS ON THE CONCENTRATION OF FREE CALCIUM IONS AS MEASURED BY THE FROG HEART METHOD[1]

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The effective concentrations of certain metal ions in body fluids are only a fraction of their total concentrations, as a result of various forms of binding. The more commonly recognized forms of complex formation, chelation and protein-binding, have been extensively studied in biological systems. The existence of a third type of binding, ion association, is suggested by physicochemical observations, but its physiological significance has not been established.

In accordance with the Debye-Hückel theory, strong electrolytes are commonly considered to be completely dissociated in aqueous solution. This theory postulates that the attraction between oppositely charged ions reduces their effective concentrations, but that this effect is a function of ionic strength and valence, and is, for the most part, independent of the specific constituents of the solution. However, multivalent ions are known to associate with each other more than can be explained in terms of this theory. These deviations are usually expressed in terms of a reversible equilibrium between free ions and "ion-pairs" formed by the association of one cation with one anion. These can not be thought of as true complexes since they probably involve only electrostatic forces. A dissociation constant can be calculated nevertheless and many have been obtained by physicochemical means (1). The extent of ion-pair formation of a given salt is poorly correlated with its solubility. For example, a highly soluble salt such as $MgSO_4$ may nevertheless undergo considerable ion-pair formation (2). It should be emphasized that the dissociation constants merely provide a convenient way of describing the behavior of the ions present, and give no definite information as to the nature of their interactions.

Since metal cations in this form are presumably ionized, and yet undissociated in the sense that they are not free in solution, it is possible that they may have physiological properties which are different from those of the free ions. For example, calcium ions, when associated electrostatically with sulfate

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ions, might no longer exhibit their characteristic actions in the body: furthermore, the electrostatic complexes themselves might have properties distinct from those of either free calcium ions or free sulfate ions. Bianchi and Shanes (3) have hypothesized that cations may be transported across the cell membrane as ion-pairs. However, direct evidence for a physiological role of ion association has not been reported.

One of the few methods available for measuring the biologically effective concentration of a metal cation is the frog heart method for ionized calcium described by McLean and Hastings (4). The effect of multivalent anions on this system has not previously been studied (except for certain observations with phosphate discussed below). The observations presented here indicate that calcium ions, when electrostatically associated with multivalent anions, are not effective in this preparation. Dissociation constants for $Ca:SO_4$, $Ca:S_2O_3$, and $Ca:HPO_4$ obtained with the frog heart method in the present study are similar to reported values obtained by measurements of solubility, conductance and pH.

METHOD

Only healthy frogs, kept for at least two months at temperatures of 12°C. were used. The frog heart was cannulated through the aorta with a cannula of 1.7 mm. external diameter containing Ringer's solution. Appropriate ligatures were placed to prevent leakage of the cannula's contents. After the heart was removed from the body, the auricle was destroyed by coagulation with a hot metal rod in order to eliminate spontaneous contraction. The ventricle was suspended in a calcium-free modified Ringer's solution at 22–25°C. The composition of this solution was constant. For each series of observations, the heart was stimulated at regular intervals with a constant voltage. The intervals varied from 2 to 5 seconds and the voltage from 30 to 100 volts, depending upon the sensitivity and contractility of each preparation. Ventricular contraction raised a glass bead which was inserted through the cannula; a wire attached to the bead elevated a lever. The contractions were thus recorded isotonically—the recording apparatus being as free of friction as possible. The amplitude was measured from an arbitrary base line on the kymograph.

Solutions to be tested were admitted to the ventricular chamber through the cannula. The heart was rinsed twice with the solution to be tested before measurements were made. The height of contractions usually became constant within a minute or so after the solution was added.

The solution used as a reference was a modification of Boyle—Conway Ringer's (5) (cf. Table I). All the solutions were equilibrated with 97 per cent O_2, 3 per cent CO_2 before use and also bubbled with this mixture while in the cannula. The final pH was 7.4 ± 0.1[3].

Each heart was first tested with standard Ringer's solution containing varying amounts of calcium (usually 0.5 to 1.6 mM. per liter). A plot of amplitude as a function of the calcium concentration was made. Then measurements were made using solutions containing multivalent anions, alternating with the reference solutions.

The solutions containing multivalent anions were prepared by substituting for the sodium chloride of Ringer's solution as follows: Na_2HPO_4 plus NaH_2PO_4 in a molar ratio of 4 to 1;

[3] No effect on the frog heart of pH changes in the range of 7.35 to 7.60 were observed by McLean and Hastings (4).

TABLE I

Composition of Ringer's Solution

	mM./L.		mM./L.
NaCl	72.2	Na^+	102.2
$NaHCO_3$	25.0	K^+	2.5
Na_2HPO_4	2.5	Ca^{2+}	1.5
KH_2PO_4	0.5	Cl^-	80.2
KCl	2.0	HCO_3^-	25.0
$CaCl_2$	1.5	PO_4*	3.0
Glucose	10.1		

Equilibrated with 3 per cent CO_2.
Osmolality, 0.22 M. pH, 7.4. Ionic strength, 0.11M.
*$HPO_4^{2-} + H_2PO_4^-$.

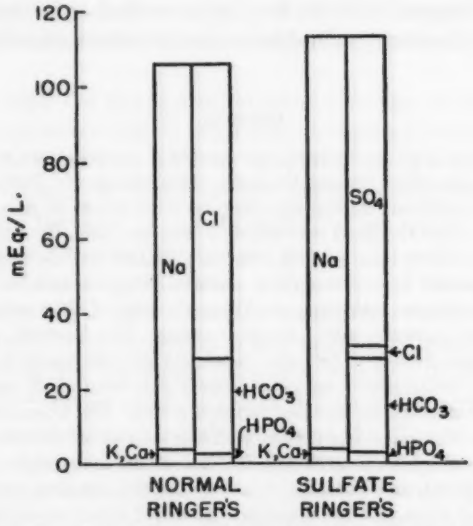

FIG. 1. The composition of normal Ringer's and sulfate Ringer's solution.

Na_2SO_4; or $Na_2S_2O_3$. In Figure 1 is shown the way this was done with sulfate. Three properties of the final solution had to be considered: ionic strength, sodium concentration, and osmolality. Osmolality was adjusted to 0.22 ± 0.01 by the addition of glucose. It is not possible to keep both ionic strength and sodium concentration constant due to the change in valence of the anions. Niedergierke and Harris (6) have recently shown that the amplitude of contraction of the frog heart varies with respect to changes in the ratio $[Ca^{2+}]/[Na]^2$. Therefore most of our observations were made keeping sodium *ion* concentration constant. The slight increase in the *total* sodium concentration in the sulfate Ringer's solution allows for the partial association thought to occur between sodium and sulfate ions (1). The ionic strength was increased from 0.11M. to 0.14M. Measurements were also made on solutions

TABLE II

Effect of Small Amounts of Multivalent Anions on Apparent Calcium Ion Concentration in the Frog Heart

Anion Added	Anion Conc.	Calcium Total	Calcium Ions
	mM./L.	*mM./L.*	*mM./L.*
SO_4	1.4	1.25	1.26
S_2O_3	1.3	1.25	1.22
HPO_4	1.0	0.70	0.65
	1.0	0.90	0.84
	1.1	1.50	1.55
$Fe(CN)_6$	0.5	1.0	>3.0
	0.9	1.5	>3.0
	1.0	1.0	>3.0

in which only half of the chloride was replaced by sulfate. In these, the change in ionic strength was correspondingly smaller. One sulfate experiment was also performed in which the ionic strength was kept constant and the sodium ion concentration varied.

In order to be certain that changes in amplitude reflect changes in calcium ion concentration, several factors had to be considered. First, it is necessary that the anions themselves not affect contractility. The addition of small quantities of sulfate, thiosulfate, and phosphate (0.5 to 1.4 millimoles per liter) had minimal effects on contraction (Table II), except in a few hearts which appeared to require phosphate for optimal contractility. Phosphate was routinely added in experiments with sulfate and thiosulfate at a concentration of 0.003M. (Table I). In studying high concentrations of phosphate, the reference solutions contained only 0.001M. phosphate. Small quantities of ferrocyanide were found to cause a marked increase in contractility, and it was therefore impossible to study calcium association with ferrocyanide by this method. A second necessary condition is that precipitation of calcium salts not occur in the test solutions. No precipitate was ever observed although the solubility product of calcium sulfate was exceeded in some cases. Calcium thiosulfate is highly soluble. In the case of phosphate, it was necessary to make all of the measurements on solutions supersaturated with respect to secondary calcium phosphate. Otherwise the change in calcium ion concentration was too small to be measured accurately. Two methods were used to avoid the precipitation of calcium phosphate. First, the addition of 0.2 mM./L. of citrate, and secondly, the use of normal serum ultrafiltrate in place of Ringer's. In these solutions, phosphate concentration could be raised to 10.0 mM./L. without visible precipitation for several hours. The effect of citrate on the apparent calcium ion concentration in these experiments is considered below. When serum ultrafiltrate was used, distilled water, $CaCl_2$ solution and sodium phosphate solution at pH 7.4 were added in such proportions that the final sodium concentration and osmolality were 0.10M. and 0.22M. respectively. The total dilution of ultrafiltrate was 25 to 33 per cent. The phosphate (7) citrate (8) and calcium (9) content of ultrafiltrate before these additions was determined analytically, and the final concentrations of these substances were calculated.

RESULTS

The amplitude of contraction as a function of calcium concentration in normal Ringer's solution is shown by the black dots in Figure 2. It should be noted that with increasing calcium concentration, the increments in amplitude

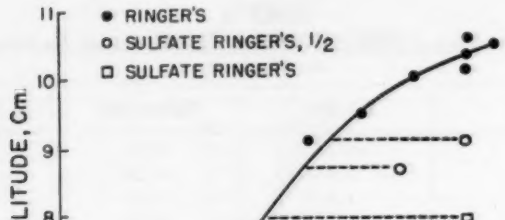

FIG. 2. The mechanical response of the frog ventricle to solutions of varying calcium concentration, with and without substitution of sulfate for chloride. [Ca]: total calcium concentration.

become smaller. Concentrations of calcium below 0.5 mM. per liter will not sustain contraction. Consequently the most reliable data are obtained at intermediate concentrations of free calcium ions.

Figure 2 also shows the effects of substituting sulfate for chloride in this experiment. It is obvious that the amplitude of ventricular contraction in the presence of sulfate is considerably smaller, at comparable concentrations of calcium. Up to one-half of the calcium in the solution is no longer effective in sustaining contraction.

Similar results were obtained with thiosulfate and phosphate. In every instance, a decrease in amplitude relative to calcium concentration in the presence of these ions was readily observed. On replacing normal Ringer's solution in the heart (Table I), the amplitude relative to calcium concentration promptly regained its former magnitude. No evidence for a progressive toxic effect on the heart was noted.

The qualitative effect of these anions is therefore clearcut. In order to express the results in a quantitative manner and to compare them to physicochemical observations, the procedure was as follows:

For each of the three electrostatic complexes, $Ca:SO_4$, $Ca:S_2O_3$, and $Ca:HPO_4$ an apparent dissociation constant, K', was obtained, as $[Ca^{2+}]\cdot[L^-]/[Ca:L]$ where $[Ca^{2+}]$ represents the concentration of free calcium ions, $[L^-]$ represents the concentration of free anions (i.e. either SO_4^{2-}, $S_2O_3^{2-}$ or HPO_4^{2-}, $H_2PO_4^-$ being neglected) and [Ca:L] represents the concentration of the ion-pair. $[Ca^{2+}]$ is obtained directly from the frog heart assay. [Ca:L] is obtained as the difference between total calcium concentration, [Ca], and $[Ca^{2+}]$ since no

other calcium ligands are present.[4] $[L^-]$ is obtained as follows:

$$[L^-] = [L] - [Na:L] - [Ca:L],$$

where [L] is the total concentration of multivalent anions added, [Na:L] is the amount of multivalent anions electrostatically paired with sodium and [Ca:L] is obtained as above.

[Na:L] is calculated from the known concentrations of sodium and multivalent anions, using published values (1) for the dissociation constants of the ion-pairs, $Na:SO_4^-$ and $Na:S_2O_3^-$, corrected to the appropriate ionic strength by the use of a standard activity coefficient equation (10). Thus, for example, the reported dissociation constant of $Na:SO_4^-$ at zero ionic strength is reported to be 0.19M. Upon correction (10) to ionic strength 0.11M. this becomes 0.564M. Since total sodium concentration, [Na], was 0.107M. and total sulfate, $[SO_4]$, 0.039M., the concentration of the ion-pair $Na:SO_4^-$ is given by:

$$0.564 = \frac{([Na] - [Na:SO_4^-])([SO_4] - [Na:SO_4^-])}{[Na:SO_4]}$$

Therefore, $[Na:SO_4^-] = 0.0059M$.

In the case of thiosulfate, $[Na:S_2O_3^-] = 0.0056M$., using a K value of 0.21 M. at zero ionic strength. In the case of phosphate, the low concentration of anion makes this calculation superfluous.

An additional complication is introduced by the fact that ionic strength was somewhat higher after the addition of multivalent anions than before, for the reasons given above. Assuming that the frog heart responds to calcium ion *activity* rather than to calcium ion *concentration*, an increase in ionic strength should diminish the response of the heart to a given calcium concentration, because the calcium ion activity coefficient would become smaller. This would occur even in the absence of specific ionic interactions. It has apparently not been shown that changes in ionic strength, independently of changes in sodium concentration (4, 6), affect the response of the frog heart. The specific effects of sodium concentration, cited above, make this demonstration difficult, and we have not attempted to pursue this problem further. Although the changes in ionic strength in these experiments were small, they were in the direction to diminish amplitude. Substitution of multivalent anions also diminishes amplitude (Fig. 2). Therefore an approximate correction for the change in ionic strength was applied, in order to be certain that the changes observed were not due to the increase in ionic strength. This was done as follows.

It was assumed that ionic strength *per se* affects amplitude only in so far as

[4] The low concentration of phosphate present in the reference solutions is negligible (Table II).

TABLE III

Effect of Sulfate on Calcium Ion Concentration

Exp. No.	[C_a]	[SO_4]	μ	$f_{Ca^{2+}}$	[Ca^{2+}]	[Ca:SO_4]	[SO_4^{2-}]	K′
	mM.	*mM.*	*M.*		*mM.*	*mM.*	*mM.*	*M.*
I	0.75	16.6	0.12	0.37	0.51	0.24	16.4	0.036
Constant [Na^+]	1.25	16.6	0.125	0.37	0.87	0.38	16.2	0.037
	1.25	33.1	0.14	0.36	0.70	0.55	32.6	0.041
	1.50	16.6	0.125	0.37	0.94	0.57	16.0	0.027
	1.50	33.1	0.14	0.36	0.76	0.75	32.4	0.033
II	1.25	16.6	0.125	0.37	0.96	0.29	16.3	0.054
Constant [Na^+]	1.25	33.1	0.14	0.36	0.80	0.45	32.7	0.058
III	1.25	16.6	0.125	0.37	0.93	0.33	16.3	0.047
Constant [Na^+]	1.25	33.1	0.14	0.36	0.77	0.48	32.6	0.052
	1.00	16.6	0.125	0.37	0.71	0.29	16.3	0.040
	1.00	33.1	0.14	0.36	0.67	0.34	32.8	0.066
IV	1.50	12.0	0.11	0.38	1.30	0.20	11.8	0.077
Constant μ	1.50	24.1	0.11	0.38	1.04	0.46	23.6	0.053
	0.75	12.0	0.11	0.38	1.60	0.15	11.9	0.048
	0.75	24.1	0.11	0.38	0.54	0.21	23.9	0.061

Mean: 0.044 + 0.012 (S.D.)

[Ca] = total calcium.
[Ca^{2+}] = free calcium ions, corrected to μ = 0.11.
[SO_4] = total sulfate corrected for pairing with sodium.
[SO_4^{2-}] = free sulfate ions.
[Ca:SO_4] = calcium sulfate ion-pair.
f_{Ca} 2 + = calculated activity coefficient of the calcium ion.
μ = ionic strength.

it affects calcium ion activity. Although this is by no means certain, as pointed out, there is no evidence to suggest a more significant effect of ionic strength on amplitude. It was also assumed that activity coefficients for the calcium ion, calculated from reported measurements of mean activity coefficients of KCl (11) and $CaCl_2$ (12) at 25°C. are valid. The apparent calcium ion concentration in each unknown solution was corrected to 0.11M. ionic strength by multiplyng by f_1/f_2, where f_1 represents the calculated calcium ion activity coefficient at ionic strength 0.11M., and f_2 represents the calculated coefficient at the ionic strength of the unknown solution. f_1 was calculated to be 0.38. f_2 varied from 0.36 to 0.37. Therefore the magnitude of this correction did not exceed 7 per cent.

In the one experiment with sulfate in which ionic strength was held constant (Table III), significant reductions in amplitude were still observed. The fall in sodium concentration would tend to increase amplitude, according to Neidergierke and Harris (6). The K′ values derived from this experiment appear

TABLE IV

Effect of Thiosulfate on Calcium Ion Concentration

Exp. No.	[Ca]	[S_2O_3]	μ	$f_{Ca^{2+}}$	[Ca^{2+}]	[Ca:S_2O_3]	[$S_2O_3^{=}$]	K′
	mM.	*mM.*	*M.*		*mM.*	*mM.*	*mM.*	*M.*
I	1.5	16.6	.126	0.37	1.28	0.22	16.4	0.095
	1.5	33.2	.14	0.36	1.19	0.31	32.9	0.126
	1.25	16.6	.125	0.37	1.10	0.15	16.5	0.120
	1.25	33.2	.14	0.36	1.03	0.22	33.0	0.154
	1.0	16.6	.125	0.37	0.89	0.11	16.5	0.133
II	1.5	33.2	.14	0.36	1.12	0.38	32.8	0.097
	1.25	33.2	.14	0.36	0.95	0.30	32.9	0.104
	1.25	33.2	.14	0.36	0.94	0.31	32.9	0.100
	1.0	33.2	.14	0.36	0.84	0.16	33.0	0.173
	1.0	33.2	.14	0.36	0.84	0.16	33.0	0.173

Mean: 0.128 ± 0.030 (S.D.)

[Ca] = total calcium.
[S_2O_3] = total thiosulfate, corrected for pairing with sodium.
[Ca^{2+}] = free calcium ions, corrected to μ = .11.
[$S_2O_3^{=}$] = free thiosulfate ions.
[Ca:S_2O_3] = calcium thiosulfate ion-pair.
μ = ionic strength.
f_{Ca} 2 + = calculated activity coefficient of the calcium ion.

to be somewhat greater, as would be anticipated; they have not been included in the averages.

The K′ values obtained for the ion-pairs Ca:SO_4 and Ca:S_2O_3 are 0.044 ± 0.012M. and 0.128 ± 0.030M. respectively.

In the case of Ca:HPO_4, the citrate added or present in ultrafiltrate required calcuation of the amount of the calcium citrate complex present in these solutions. Calcium ion concentration in the unknown solutions was obtained directly. Ionic strength remained nearly constant in these experiments with phosphate. The concentration of calcium citrate was obtained as a function of the calcium ion concentration and the total citrate (13). The amount of the ion-pair, Ca:HPO_4, was then obtained by difference.

In Table VI the mean values and standard deviations for each of the three electrostatic complexes are summarized, and compared to reported values obtained by physicochemical means. Since the physicochemical observations were made at a variety of ionic strengths, exact comparison is not possible. However, an approximate comparison can be made by correcting all the values to 0.11 ionic strength by use of a standard activity coefficient equation (10).[5]

[5] Although either the use of alternative equations or the selection of different constants in the equation employed would lead to different values for K′, the equation used herein is the same as that on which most of the physicochemical data cited in Table VI are based.

TABLE V

Effect of Phosphate on Calcium Ion Concentration

Exp. No.	[Ca]	[HPO_4]	[Cit]	[Ca^{2+}]	[$CaCit^-$]	[$Ca:HPO_4$]	[HPO_4^{2-}]	K'
	mM.	*mM.*	*mM.*	*mM.*	*mM.*	*mM.*	*mM.*	*M.*
I	1.50	10.0	0.20	0.88	0.13	0.49	9.5	0.017
	1.50	7.3	0.20	1.06	0.14	0.32	7.0	0.023
	1.50	4.6	0.20	1.04	0.14	0.35	4.3	0.013
	1.25	10.0	0.20	0.88	0.13	0.24	9.8	0.036
	1.25	10.0	0.20	0.91	0.13	0.21	9.8	0.042
	1.25	7.3	0.20	0.91	0.13	0.21	7.1	0.031
	1.25	4.6	0.20	0.99	0.13	0.15	4.5	0.029
	1.00	7.3	0.20	0.73	0.12	0.15	7.2	0.035
	1.00	4.6	0.20	0.75	0.12	0.13	4.5	0.026
II	1.04	6.7	0.10	0.8	0.07	0.17	6.5	0.030
	1.46	11.4	0.10	0.76	0.06	0.64	10.8	0.013
III	1.15	4.8	0.10	0.81	0.07	0.27	4.5	0.014
	1.64	8.6	0.10	1.05	0.08	0.51	8.1	0.017

Mean: 0.025 ± 0.011 (S.D.)

[Ca] = total calcium.
[HPO_4] = total dibasic phosphate.
[Cit] = total citrate.
[Ca^{2+}] = free calcium ions.
[CaCit] = calcium citrate complex.
[$Ca:HPO_4$] = calcium phosphate ion-pair.
[HPO_4^{2-}] = free phosphate ions.
The medium for Exp. II and Exp. III was modified dog serum ultrafiltrate.

TABLE VI

Values for K' of Calcium Ion-pairs

Ion-pair	Frog heart Mean S.D. (n)	Physicochemical Range
	M./L.	*M./L.*
$Ca:SO_4$	.039 ± .009 (11)	.030–.049
$Ca:S_2O_3$	.109 ± .025 (10)	.080–.110
$Ca:HPO_4$	.025 ± .011 (13)	.012–.030

Ionic strength: 0.11M. Temperature: 25°C.

As indicated in the Table, the resulting values derived from physicochemical measurements do not differ significantly from those obtained by the frog heart method.

DISCUSSION

These experiments indicate that multivalent anions, substituted for chloride, diminish the response of the frog ventricle to a given calcium concen-

tration. This effect can not be attributed to changes in ionic strength, sodium concentration, osmolality or pH. The increase in ionic strength was slight (from 0.11M. to a maximum of 0.14M.), and the apparent decrease in calcium ion concentration was far greater than could be attributed to non-specific interionic attraction: the latter would result in a decrease of the order of six per cent, while the observed decrements in calcium ion concentration, corrected for ionic strength, were far greater. In the one experiment with sulfate in which ionic strength was held constant (sodium concentration therefore falling), the results were similar. Osmolality and pH were held constant in all experiments. Sodium concentration, corrected for ion association with multivalent anions, was held constant in all experiments, except the one mentioned above. If sodium ion association is disregarded, the change in the ratio $[Ca^{2+}]/[Na^{+}]^2$ is less than eight per cent. Since most of the physicochemical observations include this correction, it was applied in our studies in order to facilitate comparison with these reported K's.

The possibility that the anions employed, sulfate, thiosulfate, and phosphate might alter contractility independently of an effect upon calcium ion concentration cannot be entirely excluded. However, the addition of small amounts of each anion had minimal effects on contraction (Table II), except in a few hearts which appeared to require phosphate for optimal contractility. In no case did the addition of phosphate depress the amplitude of contraction more than could be attributed to its ability to complex calcium, as indicated by the mean dissociation constant given in Table V. Large amounts of sulfate (14) and thiosulfate (15) are non-toxic in intact animals.

There is no reason to believe that chloride deficiency plays a role in these results. McLean and Hastings (4) have shown that substitution of monovalent anions for chloride did not alter the response of the heart to a given calcium concentration. Furthermore, some chloride always remained in the solutions tested (Fig. 1).

The role of solubility has been discussed briefly in "Methods". Calcium thiosulfate is highly soluble. The solubility product of calcium sulfate was only occasionally exceeded. The observed effects of these two anions therefore cannot be attributed to precipitation of their calcium salts. In the case of phosphate, the solubility product of $CaHPO_4$ (1) was regularly exceeded in the data presented in Table V. The means by which citrate delays precipitation in these solutions is obscure. However, the possibility that microcrystals of $CaHPO_4$ or hydroxyapatite were present in suspension cannot be excluded. On long standing, most of these solutions eventually became turbid.

In order to determine whether these results with phosphate were due to microcrystal formation, a series of observations was made at phosphate concentrations in the range of zero to 0.003M. No precipitation occurred in these

solutions even on prolonged standing (provided that CO_2 tension was held constant). In 30 observations, a decrease in amplitude (relative to calcium concentration) was uniformly observed. The magnitude of the change is too small to derive a reliable K′ value; however, the calculated K's (0.019 ± 0.013M.) do not differ significantly from those given in Table V. These data indicate that phosphate has an effect on amplitude which cannot be ascribed to precipitation of calcium salts, and that the magnitude of this effect is similar whether the solubility product is exceeded or not.

Further evidence that neither microcrystal formation nor specific anionic effects accounts for our results is given in Table VI. The similarity of the derived dissociation constants for these three salts to the values obtained by physicochemical means is evident. These physicochemical observations were made using a variety of methods, at ionic strengths ranging from 0.006M. to 0.2M. The use of a standard activity coefficient equation to correct these constants to 0.11M. ionic strength is of course an approximation. In some cases this was unnecessary since a few observations at ionic strength 0.1M. are available. However, the degree of uncertainty in these physicochemical constants is large, as reflected by the range of values obtained. The additional error introduced by using a standard activity coefficient equation is probably relatively slight. Therefore all of the values were compared at 0.11M. ionic strength.

Until more precise measurements become available, we may tentatively conclude that the effective concentration of calcium in the frog heart is identical with that indicated by these physicochemical constants.

Although certain observations in the literature are consistent with this conclusion, they have not been so interpreted. The effect of phosphate, for example, was studied in the frog heart preparation by McLean and Hastings (16) and subsequently by McLean and Hinrichs (17). In the absence of protein, no definite effect of phosphate was detected, but the amount of phosphate employed was small. In the presence of casein or plasma protein, a decrease in effective calcium concentration resulted from addition of phosphate in large amounts; this phenomenon was ascribed to the formation of a protein-calcium-phosphate complex, although ultrafiltrates were not prepared. In the light of the present observations, it is possible that part of this change was due to calcium-phosphate interaction, independently of protein. The addition of large amounts of sulfate (18) or ferrocyanide (19) to the fluid perfusing skeletal muscle leads to changes in electrical and mechanical responses which resemble those of calcium removal. It is likely that the formation of ion-pairs is involved.

It should be noted that the frog heart method does not measure the concentration of ionized calcium, but rather the concentration of free calcium ions. Calcium ions, when electrostatically paired with multivalent anions, are apparently not measured.

In other papers in this series, it is shown that divalent cations, when associated electrostatically with multivalent anions, are cleared at a high rate by the kidney (14) and are not measured by spectrophotometric methods for determining free divalent ions (20, 21). Considered in conjunction with the present evidence, these observations lend support to the conclusion that the effective concentrations of divalent cations in the body are influenced by ion association. Although normal plasma contains only small amounts of multivalent anions, cell fluid, urine, and abnormal plasma may contain considerable amounts. Therefore this phenomenon may be of importance in the metabolism of multivalent electrolytes.

SUMMARY

Calcium ion concentration was assayed by the frog heart method in solutions in which sulfate, thiosulfate, or phosphate was substituted for chloride. In each case a decrease in amplitude relative to total calcium concentration occurred. Evidence is presented that this effect is not due to changes in ionic strength, precipitation of insoluble calcium salts, or toxicity of the anions employed. The effective calcium ion concentration in these solutions was evidently reduced due to the presence of multivalent anions.

Apparent dissociation constants were derived from these data for the electrostatic complexes $Ca:SO_4$, $Ca:S_2O_2$, and $Ca:HPO_4$. In each case the values obtained are similar to reported values obtained by physicochemical means.

It is concluded that calcium ions, when electrostatically paired with multivalent anions, are not effective in the frog heart preparation.

ACKNOWLEDGEMENTS

We are greatly indebted to Dr. Stephen Hadju and Dr. Jean Marshall for suggestions as to methodology.

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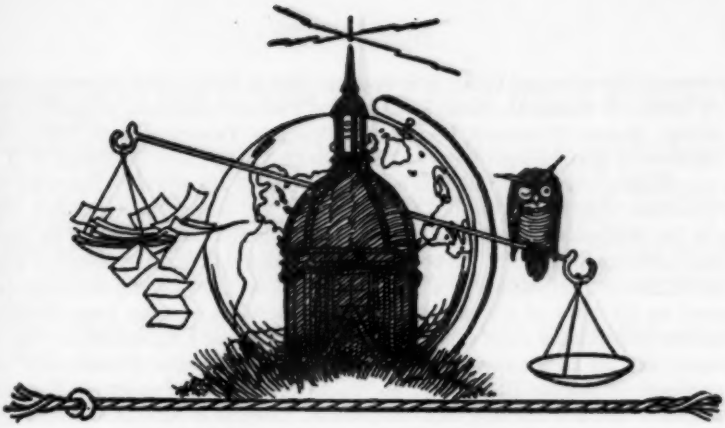

BOOK REVIEWS

Radiation Therapy. By WALTER T. MURPHY. 1041 pp., $25.00. W. B. Saunders Co., Philadelphia, Pa.

After many years of waiting, there has finally been prepared a complete text on Radiation Therapy by a single author. Dr. Murphy calls upon his twenty-five years of experience as a radiotherapist at one of the nation's leading therapy centers. This book represents the life-work of an outstanding therapist and educator. His work most certainly is destined to become a leading text in a field long dominated by European authors. His text is designed as a learning adventure for the student of radiation therapy and is a "cook book" for the radiologist who does not have the experience to establish all his own techniques. The text is well organized, presented, printed, and illustrated. It gives treatment plans in both ortho- and supervoltage and a long term supervoltage follow-up.

He presents his principal chapters in conventional fashion as follows. *Larynx:* His restriction of radiotherapy as a primary modality to Stage 0 and Stage 1 carcinomas of the larynx fails to render support to the cause of radiation therapy in this controversial field. *Breast:* This section is excellent, complete, and soundly presented. *Skin:* He advocates protracted schedules and a larger single dose, 5500 r, than is generally in use. *Lungs:* It is distressing to learn that in nineteen years of treating bronchogenic carcinoma with supervoltage generators, only three patients, treated with orthovoltage, survived five years. Quite frequently at autopsy, it is demonstrated that radiation has succeeded in destroying the intrathoracic cancer only to have the patient die of disseminated disease. *Cervix:* He uses an external radiation source as the main weapon and intracavitary radium sources to supplement the pelvic central and para-cervical dose. He uses radium in a single setting using a metallic system with intracervical sources fixed to a contra-cervical source. He gives his entire external irradiation before giving intracavitary radium treatment. This plan of therapy has many disadvantages which are well known to most radiotherapists.

The remaining chapters, devoted to the genitourinary, osseous reticulum endothelium, endothelial and central nervous system, are well presented and conform to generally accepted principles of treatment. This text is recommended to all libraries of Radiology, to the practicing radiologist, oncologist, cancer surgeon, and student of any of these specialties.

GEORGE J. RICHARDS, JR.

Microbiology Yesterday and Today. A Symposium Held in Honor of the Seventieth Birthday of Selman A. Waksman, Nobel Laureate and Professor Emeritus, Institute of Microbiology, Rutgers University, June 5, 1959. Edited by VERNON BRYSON. 122 pp., $4.00. Institute of Microbiology, Rutgers, The State University, New Brunswick, N. J.

It is a pleasure to note that this little book was not called Microbiology Yesterday, Today and Tomorrow. Only passing mention of the "Outlook" for microbiology is made on the last page by Dr. Waksman himself, a contributor to his own *Festschrift*. As might be expected, the most interesting aspects of the book concern the microbiologists of yesterday who laid the foundations of microbiology as a science. Then, as now, the leading investigators were attracted to the study of microbes from diverse disciplines, ranging from chemistry to agriculture. Of particular value as short reviews are articles by J. H. Quastel on "Microbial Chemistry and Its Development", by S. T. Cowan on "Bacterial Classification" and a superb *vignette* by Michael Heidelberger on "Episodes in Immunochemistry." Also included is a singularly dull and defensive recitation entitled "Aspects of Russian Microbiology" by G. K. Skriabin of the Microbiology Institute of the U.S.S.R. Academy of Sciences. Apparently, organizers of this symposium felt compelled to demonstrate its international flavor and in so doing fell in with Mr. Krushchev's secular doctrine of "friendly competition between nations." I should be inclined to doubt that there is a *Russian* microbiology, *per se*, or, for that matter, an Uzbek chemistry. Recognition of this fact by all scientists, including our poor neglected Russian cousins, will advance the cause of science, everybody's science.

ROBERT R. WAGNER

A History of Ophthalmology. By GEORGE E. ARRINGTON, JR. Foreword by FELIX MARTI-IBANEZ. 174 pp., $4.00. MD Publications, New York, N. Y.

This small book covers the recorded history of ophthalmology from prehistoric times to the present. The subject matter naturally and conveniently divides itself into "ages". It is especially interesting to note that each age is dominated, as a rule, by one great, creative mind, or one idea. Sometimes, as in the case of Hippocrates and Galen, the influence of an individual may dominate more than one age. Occasionally the influence is incorporated into a dogma which, far from facilitating improvement in methods of diagnosis and treatment, hinders it. In the nineteenth and twentieth centuries, according to the author, we have achieved our great growth through adherence to the idea of a free science. While suffering in places from an unwieldy prose, this book is a valuable addition to any library, documenting the coming of age of the oldest specialty in medicine.

STEWART M. WOLFF

BOOKS RECEIVED FOR REVIEW

Anesthesia for Infants and Children. By ROBERT M. SMITH. 418 pp., $12.00. The C. V. Mosby Co., St. Louis, Mo.

The Care of Minor Hand Injuries. By ADRIAN E. FLATT. 266 pp., $9.50. The C. V. Mosby Co., St. Louis, Mo.

Physiology of the Newborn Infant, 3rd edition. By CLEMENT A. SMITH. 497 pp., $12.50. Charles C Thomas, Springfield, Ill.

Principles of Disability Evaluation. By WILMER CAUTHORN SMITH. 210 pp., $7.00. J. B. Lippincott Co., Philadelphia, Pa.

The Surgeon and the Child. By WILLIS J. POTTS. 225 pp., $7.50. W. B. Saunders Co., Philadelphia, Pa.

A Synopsis of Anesthesia, 4th edition. By J. ALFRED LEE. 616 pp., $6.50. John Wright & Sons, London. The Williams & Wilkins Co., Baltimore, Md. exclusive U. S. agents.

Synopsis of Ear, Nose and Throat Diseases. By ROBERT RYAN, WILLIAM C. THORNELL and HANS VON LEDEN. 383 pp., $6.75. The C. V. Mosby Co., St. Louis, Mo.

A Synopsis of Skin Diseases. By BETHEL SOLOMONS, JR. 293 pp., $6.75. John Wright & Sons, Bristol, England. The Williams & Wilkins Co., Baltimore, Md. exclusive U. S. agents.

What Next, Doctor Peck? By JOSEPH H. PECK. 209 pp., $3.50. Prentice-Hall, Inc., New York, N. Y.

RELATION BETWEEN HEART RATE AND "SPONTANEOUS" MOVEMENTS

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It is the purpose of this paper to present our observations on the relation between heart rate and spontaneous (random) movements in the dog. Although little is known about the role played by innate behavior on cardiac activity the information to be presented suggests that some relationship exists between spontaneous movements and heart rate.

"Spontaneous" is used to indicate movements of whose origin we are ignorant, seen in a dog at rest in a closed room (camera) isolated from the external environment. These movements occur apparently as a result of some internal restlessness. They consisted specifically in sitting or standing at random and without any apparent external stimulation. The dog is ordinarily on a table, attached by a leash. Dogs were chosen who were relatively quiet who made only occasional, non-specific movements of the body, such as shifting weight, changing from sitting to standing or vice versa. The movements which we call spontaneous are not to be equated with the rhythmic spontaneous movements described by Weiss (1) in salamanders. Random is perhaps a better term owing to the connotations of the word spontaneous.

Our study supports the observations of Gantt and Hoffman (2) that an acceleration in heart rate usually *precedes* spontaneous movements in the dog.

METHODS

Five dogs, three males and two females, four normal and one neurotic were included in our experiments. These dogs were trained to stand on a table inside a sound-proof room. The movements of the dogs were observed through a one-way window.

Electrocardiograms and respiratory records were taken continuously with a Twin-Viso Sanborn machine. If the animal inside the sound-proof room assumed a sitting or standing position such motion was recorded simultaneously with the ECG and respiratory tracings. The heart rate was counted in beats per minute 12 seconds before (control) and 12 seconds after the beginning of the actual movements. Lately a Fels Cardiotachometer Model 21 A and a transducer respirometer have been used to investigate this behavior.

RESULTS

In all normal dogs the results were consistent showing an acceleration in heart rate *before* the spontaneous movements (Fig. 1). The acceleration was

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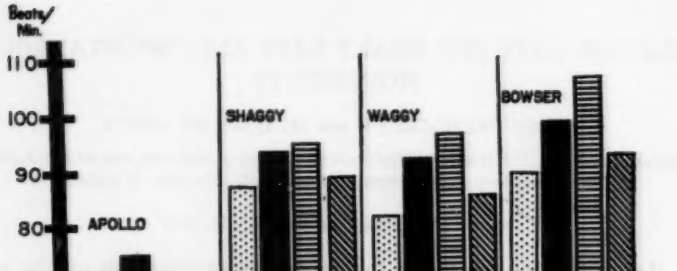

FIG. 1. Relation between heart rate and "spontaneous" or random movements in normal dogs.

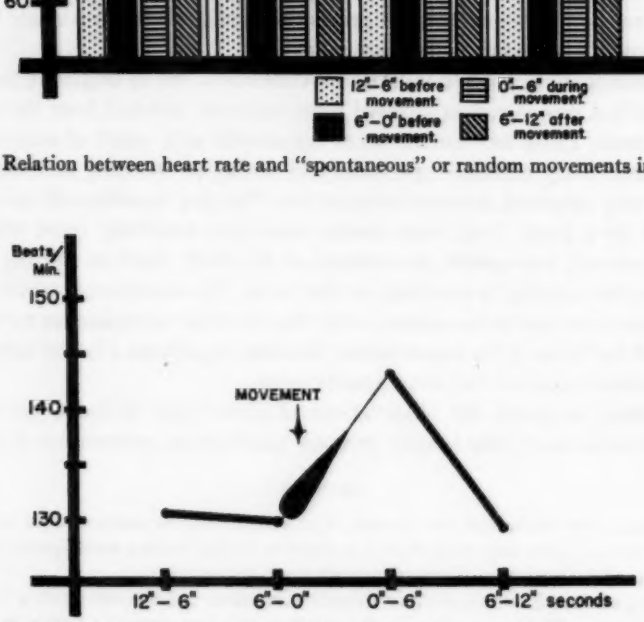

FIG. 2. Relation between heart rate and "spontaneous" or random movements in the neurotic dog V_3.

FIG. 3. Acceleration in heart rate before "spontaneous" or random movement.

usually evident 6 seconds before the movement. Contrary to what was observed in the group of normal dogs, in V_3, a neurotic dog, the acceleration in heart rate occurred *with* the movement but not before (Fig. 2). In all dogs the heart rate returned to resting levels 12 seconds after the spontaneous movement.

A graphical relationship between heart rate and spontaneous movement in a normal dog is shown in Figure 3. The tracing was obtained with a Fels Cardiotachometer. The vertical lines along the sinusoidal curve represent the beat-by-beat rate between 40 and 200 beats per minute. The artefact introduced by the spontaneous movement is represented by the irregular deflections on top of the heavy horizontal line. The heart rate increase is evident 6 seconds before the movement (arrow). Twelve seconds after the spontaneous movement the heart rate returned to the resting level.

The relation between the individual heart rates and spontaneous movements during the 12 to 6 and 6 to 0 seconds intervals before the movement in terms of acceleration, deceleration, and no change is shown in Table I. In the normal dogs the number of observations showing an acceleration in heart rate before movement is greater than the number of observations in which either deceleration or no change occurred. In the neurotic dog, however, the number of observations with no change in heart rate is greater than the number of observations with either acceleration or deceleration.

The relation between the individual heart rates and spontaneous movements 6 to 0 seconds before movement and 0 to 6 seconds during movement is shown

TABLE I

Changes in Heart Rate Before "Spontaneous" Movements

Dog	No. Cases Showing *Acceleration*	No. Cases Showing *Deceleration*	No. Cases Showing *No Change*
V_3	1	2	5
Apollo	6	0	0
Shaggy	17	5	2
Waggy	25	4	0
Bowser	13	3	1

TABLE II

Changes in Heart Rate During "Spontaneous" Movements

Dog	No. Cases Showing *Acceleration*	No. Cases Showing *Deceleration*	No. Cases Showing *No Change*
V_3	7	0	1
Apollo	1	4	1
Shaggy	12	12	0
Waggy	19	10	0
Bowser	12	5	0

in Table II. V_3 showed a definite acceleration in heart rate during the spontaneous movement but not before. In the group of normal dogs, Apollo, showed a tendency towards a deceleration during the movement. Shaggy showed an equal number of accelerations and decelerations in heart rate. Waggy and Bowser, on the other hand, showed a further acceleration in heart rate during the movement.

Table III shows the relation between the individual heart rates in the 0 to 6 seconds interval during movement and the 6 to 12 seconds interval after the movement. In all dogs the tendency is towards a deceleration after the movement ceases.

Statistical evaluation of our data was performed using the "t" test and Fisher's statistical tables (3). The level of statistical significance was set below 5 per cent. Using the test for correlated means (4) the statistical significance between the set of scores representing the heart rates 12 to 6 seconds before

TABLE III

Changes in Heart Rate After "Spontaneous" Movements

Dog	No. Cases Showing *Acceleration*	No. Cases Showing *Deceleration*	No. Cases Showing *No Change*
V_3	2	5	1
Apollo	2	3	1
Shaggy	9	14	1
Waggy	6	23	0
Bowser	2	15	0

TABLE IV

Statistical Evaluation of Data in Table I

Dog	No. Observations	σ_1	σ_2	"t"	p	Significance
V_3	8	16.7	14.0	0.304	$0.70 < p < 0.80$	Not significant
Apollo	6	2.8	1.9	7.000	$p < 0.001$	Highly significant
Shaggy	24	9.3	12.9	3.370	$0.001 < p < 0.01$	Significant
Waggy	29	8.1	10.5	3.808	$p < 0.001$	Highly significant
Bowser	17	10.3	13.7	3.905	$0.001 < p < 0.01$	Significant

No. observations represent the instances in which the dog performed a movement as described in the text.

σ_1 represents the standard deviation of the set of scores in the 12 to 6 seconds interval before movements.

σ_2 represents the standard deviation of the set of scores in the 6 to 0 seconds interval before movements.

"t" represents the "t" test for correlated means as described elsewhere (4).

p represents probability.

TABLE V

Statistical Evaluation of Control Heart Rate 12 to 6 Seconds Before Movements with that 6 to 12 Seconds After Movement

Dog	No. Observations	σ_3	σ_4	"t"	p	Significance
V_3	8	18.6	22.5	0.619	$0.50 < p < 0.60$	Not significant
Apollo	6	4.3	4.5	0.135	$0.80 < p < 0.90$	Not significant
Shaggy	24	11.4	15.0	1.071	$0.20 < p < 0.30$	Not significant
Waggy	29	10.5	9.7	1.699	$0.10 < p < 0.20$	Not significant
Bowser	17	28.2	23.5	0.729	$0.40 < p < 0.50$	Not significant

σ_3 represents the standard deviation of the set of scores in the 12 to 6 seconds before movements.
σ_4 represents the standard deviation of the set of scores in the 6 to 12 seconds after movements.

movement with the one 6 to 0 seconds before movement was compared. As shown in Table IV the increase in heart rate was significant in all normal dogs. It was not significant, however, in the neurotic dog.

Table V shows the result of the test for correlated means when we compared the statistical significance between the set of scores representing the heart rates 12 to 6 seconds before movement and 6 to 12 seconds after movement; none of the values were statistically significant, showing that 12 seconds after the movement the heart rate has returned to the resting level.

The standard deviation of the measurements before movement ($\sigma_1\sigma_2$) is numerically less than the standard deviation of the measurements after movement ($\sigma_3\sigma_4$).

The study of the respiratory changes associated with the spontaneous movements did not show any significant difference *before* the spontaneous movement. In two dogs, Apollo and Bowser, a slight diminution in the respiratory rate was observed *during* the movement.

DISCUSSION

Our experiments show that a definite acceleration in heart rate appears before spontaneous or random movements in the dog. This behavior was uniformly observed in four normal dogs but not in a neurotic dog, V_3.

The fact that the heart rate increases before the spontaneous movement seems to us to be of importance in elucidating the effect of innate behavior on cardiac activity. In the normal animal the visceral system represented by cardiac autonomic activity appears to be more immediately susceptible to changes than the more voluntary somatic system. Studying the relation between heart rate and emotional disturbances in humans, Whitehorn (5) has observed that an acceleration in heart rate occurred before movements and that the degree of acceleration bears little or no relation to the amount of physical motion in such patients.

The possibility that some degree of muscular tension may be responsible for these changes is minimized because we have observed that the peak of the acceleration comes *before* the spontaneous movement. Peters' experiments in human beings where the muscular tension is measured by a dynamometer also show that the heart rate is not proportional to the muscular tension (6). Other experiments performed in this laboratory with curare in dogs to eliminate muscular movements also support the view that the increase in heart rate during the motor conditional reflexes is due to central nervous excitability rather than to the movement itself (7). An acceleration in heart rate preceded yawning in some instances in another series of observations.

Our experiments show that a definite cardiac acceleration precedes a somatic activation known to be under the direct influence of the central nervous system. From our observations of normal behavior it thus appears as if some excitation arising within the central nervous system expressed by an increase in heart rate, not only results in but rapidly expends itself in muscular movement. With our present knowledge it is difficult to determine whether this excitation occurs in the opposite direction, that is, from the visceral structures to the central nervous system; if it does then the inner activation is the one responsible for the spontaneous movement.

In the normal animal excitation goes into the visceral system and then into movement. In the neurotic animal there seems to be inhibition of this release into the visceral system until the actual movement occurs. In the neurotic animal there is a lack of this innervation in the cardiac system until the actual movement and begins there is inhibition which prevents the release of the excitation in the way it occurs in normal dogs. Furthermore, this neurotic dog is characterized by marked inhibition extending to the muscular system resulting in a cataleptic state, as described previously (8).

SUMMARY

Our observations in isolated intact animals showed that there is an increase in heart rate *before* spontaneous movements. Paradoxically, in a neurotic dog the acceleration in heart rate occurred with the movement but not before.

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ORTHOSTATIC HYPOTENSION

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The regulation of arterial pressure depends upon complex homeostatic mechanisms (11, 20, 45, 105). In contrast to extensive knowledge of the mechanisms regulating the composition of body fluids, relatively little is known about the physiological mechanisms which enable primates to carry out their active life in an upright posture.

Beginning with the classic studies of Leonard Hill at the turn of the century, physiologists have studied the circulatory effects of the upright posture on

animals (68–70). In 1943 Hellebrandt and Franseen published a review of physiological studies of the vertical stance in man (63). The purpose of the present paper is to describe observations of a series of patients in whom abnormal circulatory homeostasis was a prominent aspect of their disease.

OUTLINE OF THE HISTORY OF ORTHOSTATIC HYPOTENSION

An early description of orthostatic hypotension was that of Piorry, the distinguished French physician, who, in 1826, was called to see a patient who had lost consciousness (106). The patient's respirations were described as irregular and stertorous. He was unconscious, and his pulse was feeble. The patient had been supported by his friends in the sitting position for fifteen minutes. Piorry refused to bleed the patient and laid him down horizontally. Immediately his eyes opened, respiration accelerated and the color came back to his face. Shortly thereafter, all the symptoms had disappeared.

In 1868, describing a patient with adrenal insufficiency, Addison wrote: "For the last month he had discontinued work on account of attacks of giddiness and dimness of sight, accompanied by a peculiar pain in the back of the head and loss of consciousness. These attacks would occur always when in the standing posture and were instantly relieved by sitting or lying down (101)."

Several years after Addison's work, Salathe pointed out that patients who had lain for a long time in bed lost the power of adapting themselves to change of position and became faint and dizzy when rising from bed (110). He first described the occurrence of syncope during convalescence, a phenomenon never thoroughly studied.

Da Costa described the condition of "irritable heart" during the American Civil War (28). Sir Thomas Lewis studied these patients with ill-defined disturbances of the cardiovascular system during World Wars I and II, and introduced the term "effort syndrome," universally adopted in the British literature, synonymous with "neurocirculatory asthenia" in the American literature (88). Three-fourths of the patients reported by Lewis complained of giddiness, unsteadiness and blurring of vision. In many patients a fall in arterial pressure of 30 to 40 mm. Hg occurred on standing.

In 1895, Leonard Hill demonstrated that snakes and domesticated rabbits were unable to tolerate an upright posture (68). He affixed a snake to a horizontal board and observed the heart directly. When the board was tilted to an upright position, the snake's heart became pale, and the animal died shortly thereafter. Hill's subsequent experiments led to his conclusion that in man splanchnic vasoconstriction compensated for the hydrostatic effect of gravity. He postulated that this "first line of defense" was supported by the "respiratory pump", that is, negative intrathoracic pressure, and by the contraction of the abdominal musculature (69, 70).

"In man a condition of deficient vasomotor tone, combined with an atonic condition of the abdominal wall or patulous abdomen, must in the erect posture lead to a deficient circulation and anemia of the brain." Hill postulated that the compensatory mechanisms were excited by the sudden fall in arterial pressure that takes place in the vertical feet-down position.

"When the vasomotor tone is in good condition, and the abdominal wall is tense, the splanchnic vessels are similar in nature to rigid tubes, and the initial hydrostatic effect of gravity is very slight. When the opposite conditions obtain, the abdominal vessels are similar in nature to an easily distended elastic bag. Thus, the whole of the blood under the influence of gravity passes therein and the circulation ceases." These initial observations, of great historic interest, have been modified and extended.

Interest in orthostatic disease increased in 1925 when Bradbury and Eggleston defined a clinical syndrome in three patients (15). This important publication provided definitive clinical criteria used subsequently to categorize a disease state consisting of:

(1) Orthostatic hypotension
(2) Syncope
(3) A fixed heart rate
(4) Heat intolerance
(5) Anhidrosis
(6) Abnormally low basal metabolic rate
(7) Elevated blood urea nitrogen
(8) Slight and indefinite signs of neurologic diseases
(9) Nocturnal polyuria
(10) Impotence
(11) Loss of libido
(12) Youthful appearance
(13) Pallor
(14) Anemia
(15) Chronic diarrhea

The previous year, Laubry had reported a patient with orthostatic hypotension that he had seen in 1891. The publication of Bradbury and Eggleston, however, laid the foundation that resulted in reports of hundreds of additional cases (15).

Bjure and Laurell (14), and Nylin and Levander (102) divided patients with orthostatic hypotension into two groups. In one, the stress on the autonomic vasoregulatory mechanisms is too great, and orthostatic hypotension results despite the occurrence of tachycardia and other compensatory mechanisms. An example is the excessive pooling of blood in varicose veins or venous angiomata. Bjure and Laurell referred to this type as "arterial circulatory anemia." Nylin and Levander used the term "sympathicotonic orthostatic hypotension." In the other group, the stress is not unusual, but the normal regulatory mechanisms are hypofunctioning or absent. Bjure and Laurell referred to this condition as "orthostatic hypotension," and Nylin and Levander described it as "asympathicotonic orthostatic hypotension."

These two classifications are presented, because they have been adopted by subsequent authors. However, as more is learned concerning the basic physiologic mechanisms of circulatory homeostasis, one can more readily classify manifestations of disease.

PHYSIOLOGIC REACTIONS TO THE ORTHOSTATIC POSITION

The evolution of mechanisms for the maintenance of an adequate cerebral blood flow has been of great importance in the transition of animals from a horizontal to an orthostatic position. Although cerebral vascular resistance decreases as the result of dilatation of intracranial blood vessels, an essential factor in maintaining adequate cerebral blood flow is adequate arterial pressure (78).

What effect does rotating the entire cardiovascular system from a horizontal to a vertical position have on arterial pressure? If the vascular system consisted of rigid tubes, the pressure differential between outflow and inflow tracts of the heart would remain constant and blood flow would be unchanged. This is analogous to a siphon. If the orifices remain in constant position, the pressure

differential and flow remain constant despite the position of the intermediate tube.

However, because of the distensibility of blood vessels, when the hydrostatic pressure increases, the vascular beds distend. The initial result is a fall in both flow and pressure in the venous outflow tracts. Thus, right atrial pressure normally decreases with the change from a recumbent to an upright position (29, 83). After the initial fall in right atrial pressure, in normal persons peripheral venous pressure immediately increases. The rise in pressure cannot be accounted for by increased hydrostatic venous pressure alone (6, 55, 104, 114). It results from reflex venoconstriction (104).

Another effect of assuming an upright position is an increase in volume of dependent parts. Grill found an increase in leg volume of 4.4 per cent (55), equivalent to an increase of 650 ml. of blood. The increased volume is the result of transudation of plasma from the vascular system. Maxfield and others found a decrease in *total* plasma volume of 4 per cent (98); Waterfield reported a decrease in whole blood volume of 15 per cent (140).

Despite the reflex venoconstriction that tends to propel blood toward the right atrium, cardiac output falls even in normal persons when the upright posture is assumed (23, 40, 57, 82, 87, 91, 125, 126). Employing right heart catheterization, Lagerlof found a 30 per cent decrease in cardiac output (83). Stead found an average decrease of 23 per cent in 7 experiments on 5 normal subjects tilted to 70 degrees (128). Scott made 129 measurements in the same individual (116). Twenty-one were during recumbency, 56 were in the sitting and 52 in the standing position. Lying, the cardiac index was 2.10 $\pm$ 0.16 (standard dev.); sitting, 1.99 $\pm$ 0.17; and standing, 1.56 $\pm$ 0.18 liters/minute.

In normal persons, the fall in cardiac output as the upright posture is assumed results in a fall in systolic arterial pressure. Lutterloh found that both children between the ages of 8 and 10 years and adults had a fall in systolic pressure and rise in diastolic pressure on assuming the upright position (93, 94). Foley found that the systolic pressure fell 1.6 mm. Hg and diastolic pressure rose 5.2 mm. Hg in 962 commercial airmen (43). Foley reviews studies of the effect of posture on arterial pressure prior to 1948 (43).

If the fall in systolic arterial pressure results from decreased cardiac output, what causes the rise in diastolic pressure? Provided the aortic valve is competent, the diastolic pressure is the force which the left ventricle must exceed to keep the aortic valve open. The level of diastolic pressure is related then to the degree of peripheral vascular resistance. Assumption of an upright posture results in increased total peripheral vascular resistance, evidenced by an increase in mean arterial pressure in the presence of a fall in cardiac output.

The vascular resistance in the arm rises in association with postural changes in a manner analogous to the total peripheral vascular resistance. If the arm is

hung in a dependant position, diastolic pressure in that arm rises, even though the subject remains supine (13).

Hill postulated that the splanchnic vasculature, highly developed in man and monkeys in contrast to four-legged animals, was the area concerned with the increased peripheral resistance associated with the upright posture (68–70). Subsequent evidence indicates that the splanchnic vessels are not the sole area concerned. The upright posture results in an increased renal vascular resistance. Brun and others found that the Diodrast clearance fell to values between 20 per cent and 45 per cent of normal in healthy individuals changing from the recumbent to the upright position (19). The mean arterial pressure did not fall. Consequently this decreased flow resulted from an increased renal vascular resistance. Smith noted a decrease in renal blood flow in the majority of normal subjects tilted to a 70–80 degree angle (123). McCann confirmed this fall in persons with nephroptosis (20 per cent to 44 per cent fall) and in normal persons (10 per cent fall) (99-a).

Pulmonary vascular resistance also increases in the upright position (122). In studies of two normal persons, Lagerlof found that the pulmonary vascular resistance doubled when the subjects assumed an upright posture (83). Similarly, vascular resistance in the extremities increases when the upright posture is assumed. This has been shown by Page and others to be related to venoconstriction (104). Hepatic blood flow is also reduced during quiet standing (16).

Thus, all vascular beds studied, with the exception of the cerebral vascular bed, show evidence of increasing vascular resistance and decreasing blood flow associated with the assumption of the upright posture. Cerebral vascular resist-

TABLE I

Principal Cardiovascular Consequences of Assuming the Upright Posture

Increased intraluminal hydrostatic pressure in blood vessels below the heart
Transudation of plasma outside the blood vessels
Reflex arteriolar constriction in pulmonary, renal, splanchnic, hepatic and extremity vascular beds
Increased peripheral vascular resistance and decreased blood flow in these areas
Reflex venoconstriction
Decreased cardiac output
Increased cardiac rate
Decreased systolic arterial preasure
Increased diastolic arterial pressure
Increased mean arterial pressure
Decreased cerebral vascular resistance
Increased tissue pressure in the legs
Pumping action of the leg muscles forcing blood past venous valves
Increased tension in abdominal muscles
Release of antidiuretic hormone of the posterior pituitary
Release of aldosterone from the adrenal cortex
Release of norepinephrine from sympathetic nerve endings and adrenal medulla

ance decreases, probably as an effect of humoral agents and local metabolites, rather than neurogenic factors (78).

Another circulatory change is an increased pulse rate. The usual response to assuming the upright posture from recumbency is an increase of about 10 beats per minute (6, 59, 62, 100, 117, 130).

Other circulatory changes that occur are an increased tissue pressure in the legs (99), a pumping action of the leg muscles forcing blood past the venous valves (63, 65, 99), reflex hyperpnea that increases the duration of the period of negative intrathoracic pressure (44), and increased tension in the abdominal musculature (69, 70). These changes produce an increased venous return to the heart.

Assumption of the erect position results in the release of certain hormones. The output of the antidiuretic hormone of the posterior pituitary is increased in the erect posture (19). It is also likely that both norepinephrine and aldosterone secretion are increased in the erect posture (8, 38, 100b). Table I summarizes the physiological consequences of assuming the upright posture.

UNUSUAL VASOREGULATORY STRESS IN NORMAL PERSONS

The occurrence of orthostatic hypotension in normal persons is unusual. Foley found no instances of orthostatic hypotension in 1,154 normal persons, primarily commercial airline pilots and crewmen, who stood erect following a short period of recumbency (43). In contrast to actively standing, passive tilting from the recumbent to upright posture in many normal persons leads to syncope (3). With passive tilting, muscular contractions of the legs and abdomen and increased tissue pressure do not occur (62). Under certain other conditions, normal persons will develop orthostatic hypotension.

Gravitational stress

In 1926, Bauer first described the phenomenon of "dim-out" and "blackout" (10). He described the result of centrifugal forces on a pilot's body when coming out of a dive or making a sharp turn in an airplane.

"The blood is carried away from the head to his lower extremities and especially into the easily dilated splanchnic vessels. Anemia of the brain, resulting in haziness, dizziness and even unconsciousness results."

Further studies, chiefly those of von Diringshofen in 1932 (33), and Jongbloed and Noyons in 1933 (77), expanded these concepts. They found that the normal human subject could tolerate about a fourfold increase in the usual gravitational force by means of physiological adaptations. Most human beings lost consciousness after being subjected to a 5g acceleration for a period of six to seven seconds. Studies of excessive gravitational stress are numerous and consequently will not be considered in detail.

Thermoregulatory stress

Patients with febrile illnesses frequently have orthostatic hypotension. Under experimental conditions, 50 per cent of normal persons develop syncope when they assume an upright posture after twenty minutes immersion in a bath of temperature 40 degrees centigrade (71). The persons who faint are able to tolerate the upright posture for about five minutes. During this time the diastolic pressure rises, presumably as a result of increased peripheral resistance. The systolic pressure falls, a result of the fall in cardiac output. After this period of normal compensation, the patients decompensate and manifest apprehensiveness, nausea, light-headedness, yawning, deep breathing, pallor, bradycardia, and a fall in mean arterial pressure.

A similar response may occur during artificial fever therapy for syphilis (79). Occasionally in the course of induced fever, a change in the position of the patient from the recumbent to the sitting position results in marked weakness, fainting and even unconsciousness and convulsions. In some of these patients, adaptation is insufficient immediately after the assumption of the upright position. In others, initial compensation is good, but as the position is maintained, adaptation becomes inadequate, and syncope results.

Exertional Stress

In 1930, Jokl described "Sportkrankheit", or post-exercise circulatory collapse (76). Additional studies were made by Mateef in 1936 (97), and by Eichna, Horvath and Bean in 1947 (36). Orthostatic hypotension developed in approximately 50 per cent of normal young men following vigorous exercise of the lower extremities. Of the subjects who developed orthostatic hypotension, syncope occurred in half. The violent exercise resulted in peripheral vasodilatation, perhaps a direct effect of carbon dioxide accumulation or as a neural reflex, a mechanism suggested by Sharpey-Shaeffer in 1958 (118).[1]

PATHOLOGIC REACTIONS TO THE ORTHOSTATIC POSITION

Cardiac output and peripheral vascular resistance

The usual response of normal persons to the upright posture is a slight fall in cardiac output associated with arteriolar constriction and a consequent rise in diastolic arterial pressure. The fall in cardiac output in patients with orthostatic hypotension is exaggerated. Hickam and Prior analyzed the results obtained in three patients with idiopathic orthostatic hypotension, and in nine patients with other types of orthostatic hypotension (67). In contrast to the results obtained in normal persons in whom the mean fall of cardiac output is approximately 25 per cent and the increase in peripheral vascular resistance about 33 per cent, patients with orthostatic hypotension had a fall in cardiac

[1] It is possible that the "effort syndrome" occurs in those persons who have an abnormally low threshold of resistance to exertion.

output of greater than 40 per cent. These patients had only a slight increase in peripheral vascular resistance or even a fall in some cases. The orthostatic hypotension is the result of inadequate peripheral vascular resistance in the upright position, intensified by an excessive postural fall in cardiac output.

Venomotor activity

In addition to the deficient arteriolar constriction on assuming the upright position, venomotor activity is abnormal in these patients (104). In normal persons, stimulation of the sympathetic nervous system results in venoconstriction, an increase in venous pressure, and a decreased capacity of the venous system, thereby forcing a large amount of blood out of the veins and into the right atrium. An important function of the veins is their ability to increase or decrease venous return to the heart and thereby regulate cardiac output (58). For example, compression of the abdominal veins decreases the venous return to the heart and arterial pressure falls markedly. This may be observed when a pregnant woman near term lies supine (72).

Abnormal venomotor activity in persons with orthostatic hypotension may be demonstrated in several ways. In normal persons, vigorous reflex venoconstriction results from the Valsalva maneuver, tilting, exercise, cold and hyperventilation. Patients with orthostatic hypotension show very little increase in venous pressures in response to these same stimuli (104). The loss of reflex venoconstriction is equally as important as defective arteriolar constriction.[2]

In 1941 Stead and Ebert suggested that arteriolar vasoconstriction was defective in persons with idiopathic orthostatic hypotension. They demonstrated that the blood flow in the fingers and hands did not decrease as much in patients with orthostatic hypotension as in normal persons with a comparable fall in arterial pressure (127). Similarly, Jeffers and his collaborators concluded that reflex vasoconstriction was a primary defect in orthostatic hypotension (74). Verel arrived at similar conclusions, although he did not find an abnormally reduced blood flow in the extremities (131).

Heart rate

When Bradbury and Eggleston first described the syndrome of orthostatic hypotension, they emphasized the constancy of the pulse rate despite the fall in arterial pressure (15). However, an unchanging heart rate is not a *sine qua non* of the syndrome. The changes in heart rate are related to the severity of the disease. A patient reported by Verel and one patient in the present series were observed to develop a fixed heart rate as the disease progressed (131). When first examined, both patients had a marked increase in heart rate in

[2] A transiently decreased venous tone with a failure of the ejection into the heart of a sizable volume of blood in response to venoconstriction may possibly play a role in the orthostatic hypotension of transient type, e.g. following a hot bath or in persons with poor muscular tone.

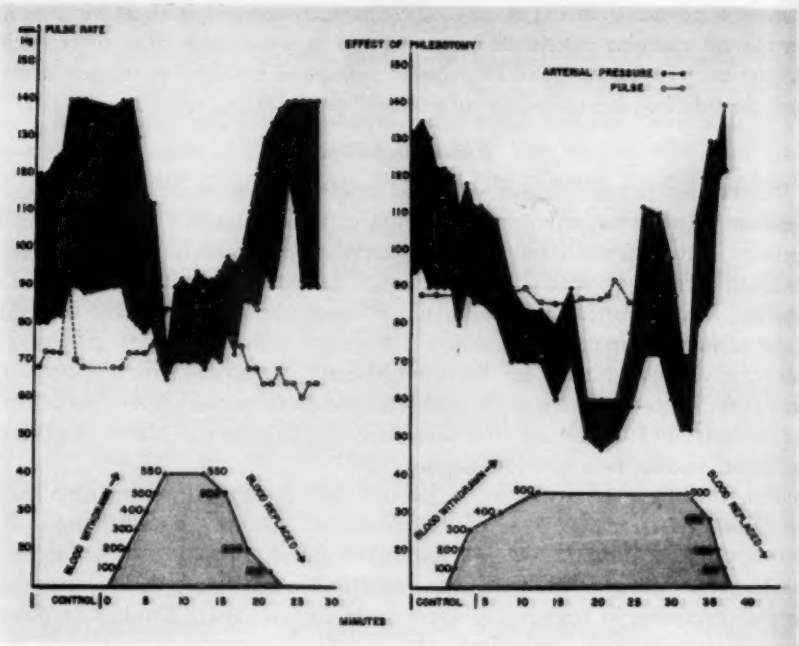

FIGURE 1. Effect of withdrawing and subsequently reinfusing 500 ml. of blood on the arterial pressure and pulse rate of patients with orthostatic hypotension. The patients were recumbent throughout the study.

association with the fall in arterial pressure. The heart rate failed to accelerate as the disease progressed to the extent that the cervicothoracic neural dermatomes became involved.

Correlation between the extent of involvement and the failure of the heart rate to accelerate may also be observed in patients who have had paravertebral sympathectomy. Total sympathectomy as performed by Grimson results in a nearly constant heart rate (56), whereas low thoracolumbar sympathectomy results in orthostatic hypotension with compensatory tachycardia (49). The fact that exercise, carotid sinus massage, atropine administration, and immersion of the hand in ice water do not affect the heart rate also indicates that cardiac accelerator nerves are involved in severe cases of orthostatic hypotension, whether idiopathic, tabetic or diabetic.[3] In adrenal insufficiency, hypovolemia and the orthostatic hypotension of infection, the heart rate usually increases markedly because the autonomic innervation of the heart remains intact. One must be aware, however, that in about one half of the idiopathic

[3] Although carotid sinus pressure is without effect in 30 per cent of normal people, it is almost uniformly ineffective in patients with severe orthostatic hypotension (141).

cases, cardiac accelerator nerves may be spared, and the heart will accelerate when arterial pressure falls. Occasionally, a marked tachycardia may occur in the upright position. Paroxysmal auricular tachycardia (41) and paroxysmal auricular fibrillation (143) have been reported to occur upon standing.

Response to volume changes

The fall in arterial pressure that occurs when patients with orthostatic hypotension assume an upright posture is a specific manifestation of a general inability to maintain stability of the arterial pressure in response to stimuli tending to change it (133). Figure 1 illustrates the effect of hemorrhage on arterial pressure in two patients with orthostatic hypotension. As blood was withdrawn from the patients in the recumbent position, a marked fall in arterial pressure occurred. When the blood was reinfused, the pressure returned to normal levels.

Arterial pressure increases markedly when blood volume or extracellular fluid volume is suddenly expanded by the rapid infusion of whole blood, normal saline, or albumin (133). These experimental results, obtained with the patients recumbent, are in contrast to those obtained in normal persons. An

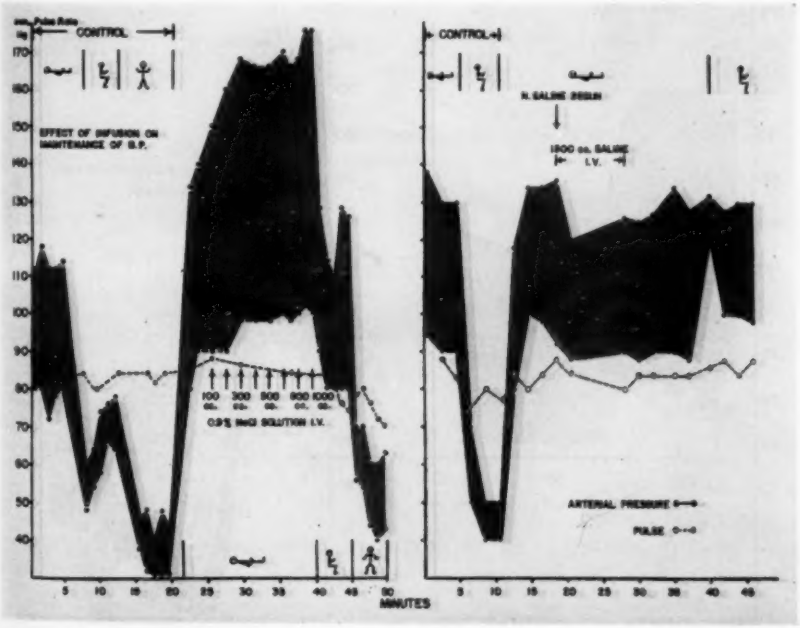

FIGURE 2. Effect of expansion of the extracellular fluid volume with 0.9 per cent NaCl solution. Different positions of the patients during the study are illustrated in the figurines. The point of infusion is indicated by arrows.

example of the effect of normal saline infusion on the arterial pressure of a patient with orthostatic hypotension is shown in Figure 2. The arterial pressure rose to supranormal levels following the saline infusion, and the fall in arterial pressure was significantly less when he assumed a sitting position.

Response to vasoactive drugs

That the defect is a generalized inability to maintain circulatory homeostasis may also be demonstrated by the increased reactivity of patients with orthostatic hypotension to vasoactive substances of diverse types, both pressor and depressor (134). For example, vasopressin and norepinephrine cause marked hypertension, and oxytocin and nitroglycerine marked hypotension. The response of two patients to nitroglycerine (0.6 mg. sublingually) is shown in Figure 3. The response to norepinephrine (2 micrograms intravenously) and to tetraethylammonium chloride (400 mg. intravenously) is illustrated in Figure 4. Hyperactive responses to the pressor substance, vasopressin, and the depressor substance, oxytocin, are shown in Figures 5 and 6.

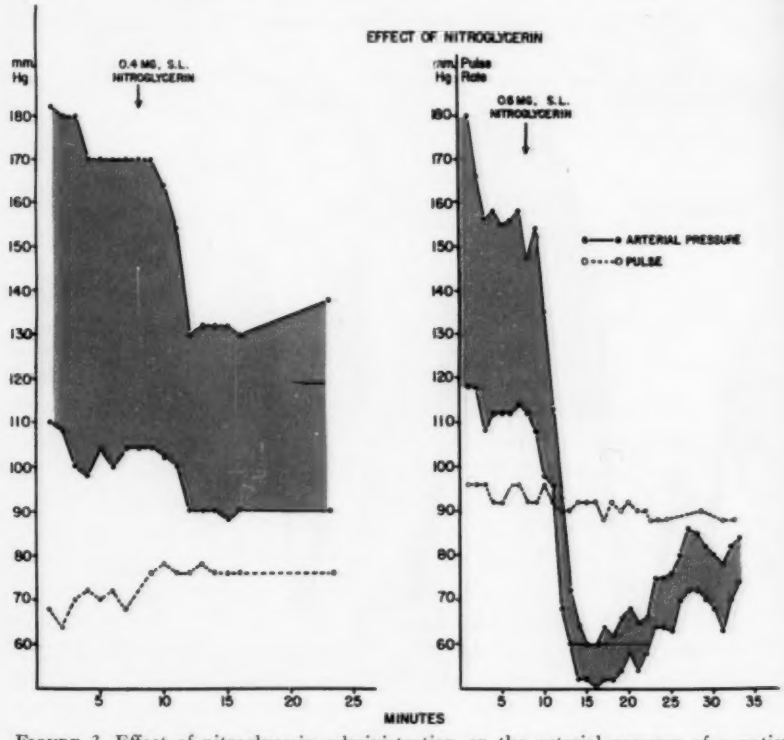

FIGURE 3. Effect of nitroglycerin administration on the arterial pressure of a patient with orthostatic hypotension.

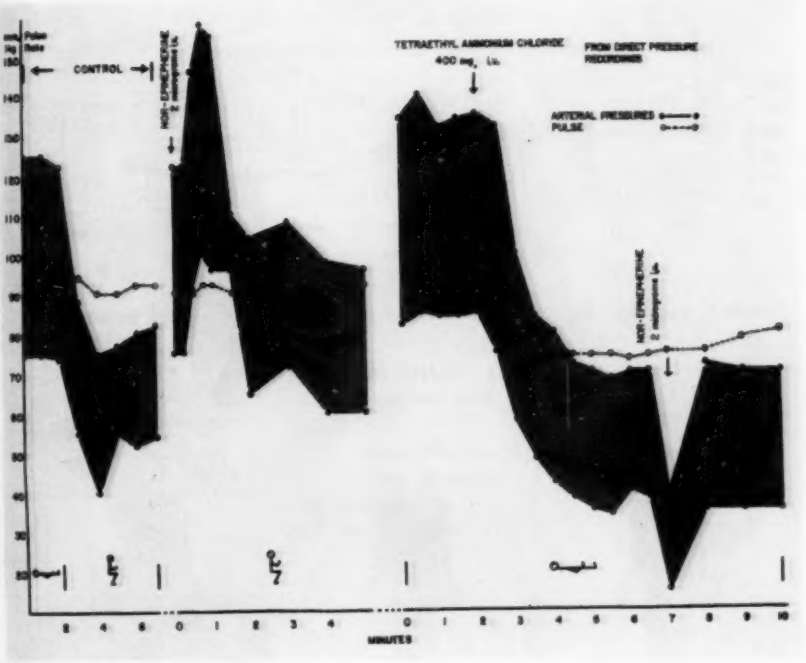

FIGURE 4. Effect of norepinephrine and tetraethylammonium chloride on the arterial pressure of a patient with orthostatic hypotension.

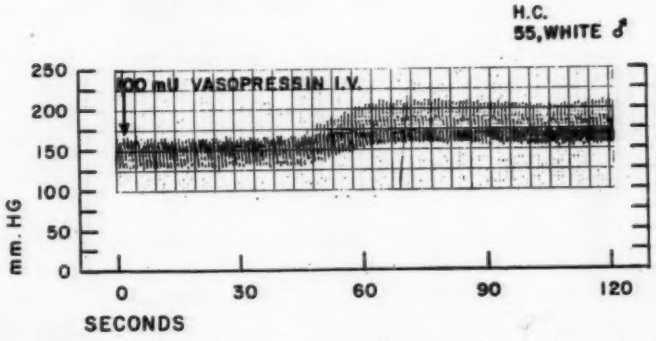

FIGURE 5. Effect of vasopressin on the arterial pressure of a patient with orthostatic hypotension.

Excretion of neurohormones

The urinary excretion of norepinephrine is decreased in patients with orthostatic hypotension (8, 38, 67b). Likewise, the stimulating effect of insulin-induced hypoglycemia on the urinary excretion of norepinephrine is markedly

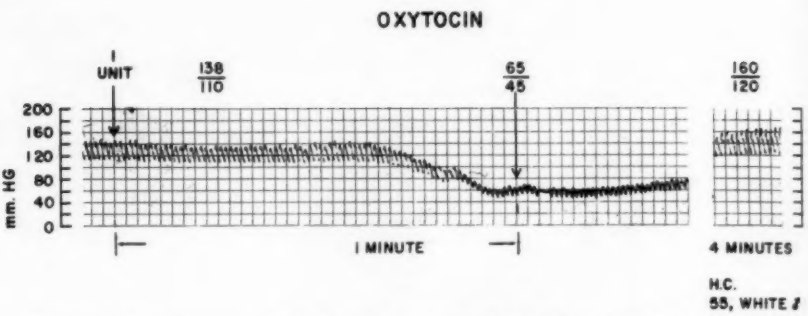

FIGURE 6. Vasodepressor effect of oxytocin on a patient with orthostatic hypotension.

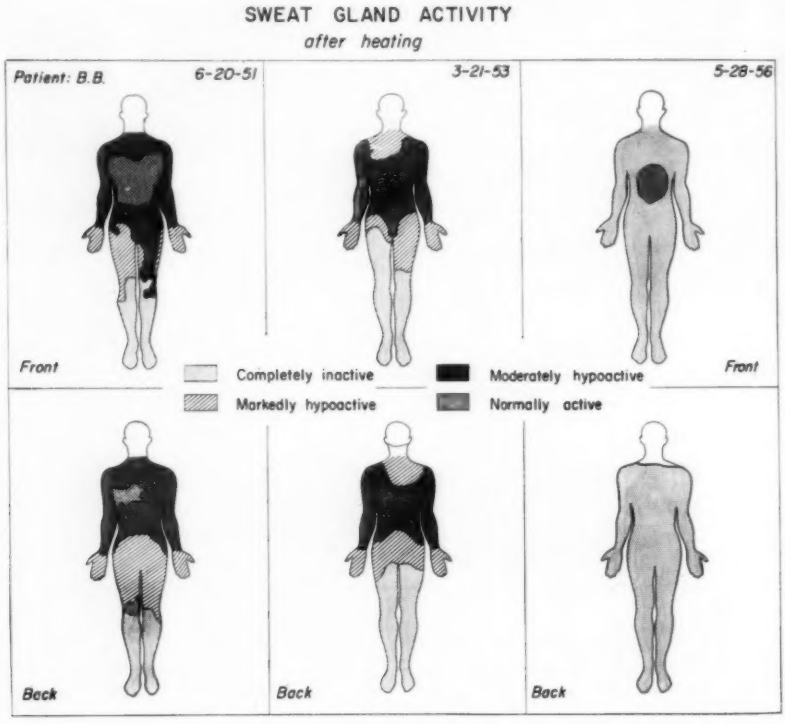

FIGURE 7a

FIGURE 7 a, b. Progression of the sweating defects over a period of five and nine years, respectively, in two patients with orthostatic hypotension. The initial glove and stocking pattern progressed to involve nearly the whole body. These sweating patterns were measured by the electrical skin resistance method.

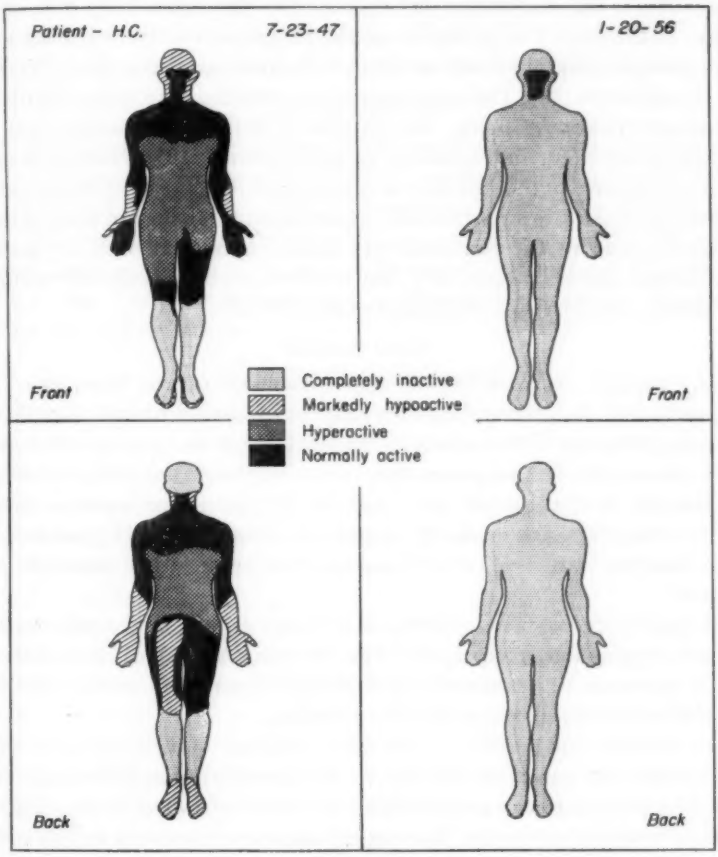

FIGURE 7b

impaired. The same degree of hypoglycemia that results in a tenfold increase in healthy subjects had no effect in two patients with orthostatic hypotension.

A defective secretion of the antidiuretic hormone (ADH) could not be demonstrated in patients with orthostatic hypotension. This is of interest since some evidence indicates that the assumption of the upright posture stimulates ADH release in normal man (19).

The excretion of a urinary vasodilator substance, possibly a substance of large molecular weight, called callicrein, is diminished in patients with orthostatic hypotension (112).

Sweating defects

Sweating defects occur in about one-third of the patients (124). The defect usually affects the extremities, but may involve the entire body with the exception of the face. Two examples of the progression of the sweating defect over a period of time are shown in Figure 7. In an occasional patient, the defect may be unilateral (48). The usual pattern is a stocking and glove distribution with symmetrical progression. The pattern of the defect simulates that seen following paravertebral sympathetic ganglionectomy (103). Normal sweating occurs in response to the injection of pilocarpine. The sweating defect may be observed in patients with orthostatic hypotension secondary to tabes dorsalis, or diabetes, and in the autonomic neuropathic group. It does not occur in patients with adrenal insufficiency, but occurred in one patient with panhypopituitarism secondary to a craniopharyngioma (132).

Renal function

Many patients with orthostatic hypotension have urea retention. Those with associated diabetic nephropathy may have marked uremia. Renal blood flow and glomerular filtration rate in most patients with the autonomic neuropathic disease are normal when they are recumbent, but fall markedly on assuming the upright posture (24). Sodium and potassium excretion fall (4) and the urine becomes markedly hypertonic despite serum hypo-osmolality (132). Maximal rates of tubular reabsorption or secretion have not been measured.

Nearly all patients have a reversal of the normal pattern of urine excretion, manifest by polyuria at night and oliguria during the day. This results from the low glomerular filtration rate and possibly from ADH release associated with the low arterial pressure of daily activities.

These patients and normal persons differ strikingly in their ability to excrete both sodium and water in response to the administration of isotonic saline (133). The patients have a greatly enhanced ability to excrete both sodium and solute-free water when supine. This suggests that the presence of well-developed vasoregulatory reflexes functioning normally to maintain circulatory homeostasis have resulted in normal man's relative inability to rapidly excrete excesses of salt.

Aldosterone excretion

In several respects, patients with idiopathic orthostatic hypotension resemble patients with Addison's disease. They have, in addition to orthostatic hypotension, generalized weakness, easy fatiguability, gastrointestinal disturbances, impotence, nocturnal polyuria, and a relative inability to remain in sodium balance when dietary sodium is restricted. None has abnormalities of skin or mucous membrane pigmentation or hypoglycemic episodes.

Because of the similarity, an investigation was made of the urinary excre-

tion of aldosterone in response to a low sodium diet in normal persons and in persons with orthostatic hypotension (136). Patients with orthostatic hypotension had a subnormal urinary excretion of aldosterone and an abnormally persistent negative sodium balance when sodium intake was restricted. Hydrocortisone plasma levels and urinary excretion rates were within normal limits.

Cerebral blood flow

Cerebral blood flow was measured during recumbency in three patients with orthostatic hypotension by the nitrous oxide method (138). After basal cerebral blood flow was determined, arterial pressure was elevated by the infusion of whole blood removed from the patients one week prior to study. As arterial pressure increased, cerebral blood flow increased. The increase in blood flow was less than the pressure increment, indicating a concomitant increase in cerebral vascular resistance.

Electrocardiographic changes

In this series of patients with autonomic neuropathy, as well as in those reported by others, remarkably slight alterations in the electrocardiogram occurred in association with the fall in arterial pressure (115). This is in marked contrast to the depression of ST segments and the inversion of T waves that occur in the orthostatic hypotension of convalescence, post-exertional orthostatic hypotension, and other types in which autonomic nervous system activity is marked.

Ewert has emphasized the presence of ST-T changes in the "sympathicotonic" patients and the absence of such changes in the "asympathicotonic" group (39). He concluded that the T wave inversion of shock, exercise, and anemia may be the result of sympathetic nervous system activity rather than anoxia.

Grant and Dodge have found that in young persons, rather marked T wave inversion occurs when they are passively tilted to the upright position (53). These changes are not observed in older normal individuals or in patients with idiopathic orthostatic hypotension.

Hammarstrom, in a study of the electrocardiogram before and after sympathectomy, found considerable evidence that sympathectomy may abolish T wave inversions in coronary artery disease (60).

Gastrointestinal defects

Anorexia is present in some patients. In many, either persistent constipation or diarrhea, or alternating constipation and diarrhea are observed. In those with diarrhea, weight loss is occasionally extreme.

The only significant physiologic study of the gastrointestinal tract that has been done is a study of the effect of insulin-induced hypoglycemia (137). Not

only does the hypoglycemia fail to induce a compensatory increase in pulse rate and an associated increase in blood sugar, but also the usual increase in gastric acidity and volume of gastric juice does not occur. This provides evidence that vagal innervation is deficient in some patients. Unfortunately, conclusive intestinal motility or absorption studies are not available.

CLASSIFICATION OF ORTHOSTATIC HYPOTENSION

Endocrinopathic orthostatic hypotension

The first disease in this category is adrenal insufficiency. Addison himself noted orthostatic hypotension in adrenal insufficiency (101). A possible mechanism is that the responsiveness of the vascular bed to norepinephrine in adrenal insufficiency is diminished (47, 108). The mild postural hypotension of panhypopituitarism and hypothyroidism presumably results from a similar defect (95, 107, 113).

Diabetes mellitus is another endocrinopathy important as a cause of orthostatic hypotension (96). Berner compared a series of diabetic patients who had nephropathy with a group that had neuropathy (12). Of the patients with neuropathy, manifest chiefly by sphincter disturbances, impotence, pupillary disturbances, pareses of extraocular muscles, pains in the extremities, paresthesias, and disturbed sweating, eight of thirty-two had orthostatic hypotension. No patients with nephropathy alone had orthostatic hypotension. All eight patients had a significant increase in pulse rate in association with the orthostatic hypotension, indicating that the cardiac accelerator nerves were not involved.

Rundles studied diabetic neuropathy extensively (109). One in twenty diabetic patients complained of heat intolerance, weakness, fainting spells, bouts of tachycardia, unusual gastrointestinal disturbances, or distressing genitourinary symptoms. Twelve diabetic patients with orthostatic hypotension had evidence of neural involvement.

Odel, Roth and Keating noted the similarity of the sweating abnormalities in diabetic neuropathy with the defects observed after paravertebral sympathectomy (103). These authors found orthostatic hypotension in 19 of 20 patients with diabetic retinopathy. Only four had a tachycardia when arterial pressure fell.

An illustrative case history follows.

J. O. (WRAMC #3972 052) was a 69 year old retired army sergeant. His mother had diabetes mellitus. Five years prior to admission to Walter Reed Army Medical Center in November 1955, he noted a decrease in the urinary stream and nocturia. Two years prior to admission, he developed paresthesias of the feet, ankles, and fingertips, heat intolerance, decreased exercise tolerance, generalized weakness, and occasional blurring of vision and lightheadedness, usually precipitated by standing. On physical examination, arterial pressure was 215/100 mm. Hg supine and fell to 150/80 after several minutes of passive standing.

There were hemorrhages, exudates and arteriolar changes in the ocular fundi. The pupils reacted poorly to light. There was diminished touch, pain and temperature sense below the midcalf bilaterally. Laboratory data of significance were: Hematocrit 35 to 39 per cent, persistent albuminuria and glucosuria, negative serological test for syphilis, PSP excretion 57 per cent in 2 hours, BUN 20 mg. per cent. Chest x-ray and electrocardiogram indicated left ventricular hypertrophy. Ophthalmological examination revealed a superior temporal hemianopsia. During this hospitalization, he required 20 units of NPH insulin daily. Ankle edema and congestive heart failure developed and he was digitalized.

In November 1956, he was readmitted to Walter Reed Hospital with dyspnea on exertion, orthopnea and two-plus pitting ankle edema. At that time, he stated that he had had chronic fatigue and paresthesias of the extremities for 10 years. He complained of loss of potentia of two years duration. Arterial pressure was 196/94 in the sitting position, falling to 94/60 after 15 minutes of quiet standing. Liver was three fingerbreadths below the costal margin. The deep tendon reflexes were active and equal. Sensation to pinprick was absent in both feet and lower legs. Vibratory sense was diminished. Laboratory data were: Hematocrit 27 per cent, BUN 44 mg per cent, (30 to 63 mg per cent), PSP 28 per cent and 21 per cent in two hours, three-plus albuminuria.

During this hospitalization, orthostatic hypotension without an associated increase in pulse rate was repeatedly demonstrated. Sweating was absent over the entire body (Axillae and face not specifically tested).

Potassium depletion

In experimental animals, potassium depletion is associated with hypotension (45a, 46a). An illustrative case of potassium depletion with orthostatic hypotension is the following patient:

R. W. (J.H.H. #726855) was a 56 year old Negro who complained of dizziness, weakness and epigastric distress for several years. For 10 years he had had an enlarging non-toxic goiter. He had lost his energy and had a poor appetite with occasional nausea. Once, following straining at stool, he developed a profuse cold sweat, palpitations and syncope. He had occasional episodes of dizziness and weakness on exertion. He gradually became impotent. His local physician treated him for microcytic hypochromic anemia. A year later, however, his symptoms recurred. He had a poor appetite, lost weight and became weak. He had a syncopal attack on one occasion after blowing his nose. Several times he fainted on exertion. He had had no sweating abnormalities. For a long period of time he had taken laxatives and had had 2 to 3 watery bowel movements per day, amounting to 500 to 1000 ml. volume. On examination, his pressure fell from 116/60 in the supine position to 40/30 and finally became unobtainable on standing. Pulse rate increased from 78 to 108. Pupils were normal. Ankle jerks were absent. His hematocrit was 45, white blood count 15,000. Urine was acid and contained a trace of albumin. Urine specific gravity varied from 1.007 to 1.015. His non-protein nitrogen was 120 mg per cent. Serum bicarbonate was 35.1 mEq./L., chloride 76.2 mEq./L., potassium 1.8 mEq./L., sodium 123 mEq./L., and phosphorus 6.8 mg per cent. Kidney biopsy revealed abnormal hyalinization of glomeruli and vacuoles in the tubular epithelium. Initially the patient's arterial pressure did not respond significantly either to vasopressin or norepinephrine. After potassium repletion, there was a 50 mm. Hg increase in arterial pressure and a compensatory bradycardia when 5 micrograms per minute of norepinephrine were infused. Ten times this amount had previously failed to give a pressor response. His uremia disappeared with potassium repletion. With potassium repletion and correction of the extracellular fluid volume deficit, the postural symptoms disappeared. His hematocrit fell from 45

to 29 per cent, evincing both anemia and sodium deficiency on admission. On barium enema, there was evidence of colitis, thought to be induced by the extensive use of laxatives. When examined eleven months after discharge, the patient was entirely well except for a substernal thyroid that was surgically removed without difficulty.

Amyloidosis

Amyloidosis has been shown to be a cause of orthostatic hypotension. The following patient is an example.

M. K. (NIH #011665) was a 54 year old professor of agriculture who was admitted because of the nephrotic syndrome. This was diagnosed two months prior to admission when he noted swelling of the ankles. Shortly before admission he developed a diffuse, red, papular rash over the trunk and face. Except for this finding and edema of the legs and sacrum, physical examination was normal. Proteinuria, hypercholesterolemia and marked hypoalbuminemia were present. There were bilateral pleural effusions on x-ray examination. The patient was treated with adrenal steroids, had a marked diuresis, and was discharged. He returned after a few weeks because of nausea and vomiting. He had begun to have weakness and dizzy spells on standing. On readmission, his arterial pressure was 88/74 and pulse 98. Slight pitting edema of the legs was noted. During this hospitalization, the patient had frequent syncopal attacks and associated vomiting episodes. He had a persistent tachycardia. The pulse rate was in the range of 120 almost constantly, even when recumbent. Blood Wasserman and L.E. cell preparations were negative. Urinary oxysteroid excretion before and after ACTH infusion were normal. Blood urea nitrogen was between 68 and 58 mg. per cent. The postural hypotension, manifest by a fall in arterial pressure to 60/40, was not improved by intravenous albumin infusion, norepinephrine, or vasopressin infusions. Renal biopsy revealed typical amyloidosis. The patient continued to have tachycardia and hypotension, aggravated by the sitting position. He developed congestive heart failure. His uremia progressed and he died in coma. Extensive amyloidosis was found at autopsy.

Hypovolemic orthostatic hypotension

One frequently observes orthostatic hypotension in patients who are anemic especially when the anemia results from acute hemorrhage (50, 51). Patients with hypovolemia resulting from dehydration and electrolyte loss may also have orthostatic hypotension.

Central neuropathic orthostatic hypotension

Orthostatic hypotension is a common finding in patients with tabes dorsalis. In addition to being the first person to note this correlation, Strisower observed in 1931 that the heart rate in tabetic patients frequently failed to accelerate when arterial pressure fell (129). In seventeen patients with tabes dorsalis examined by Ellis and Haynes, ten had an abnormal response of arterial pressure to postural changes (37). Two had definite postural hypotension; the others had a somewhat greater than normal drop in the systolic arterial pressure and a slight fall in the diastolic pressure. One tabetic patient had failed to sweat for 8 to 10 years. An unusual case of tabes dorsalis with orthostatic hypotension was

that of Fine and Miller (41). This 16 year old girl had Hutchinson's teeth, Argyll Robertson's pupils, a positive serological test for syphilis and a paretic colloidal gold curve. Of particular interest, in addition to her age, was the finding that she had a marked tachycardia. Even when she was recumbent, her pulse was in the range of 120. When she sat, the heart rate increased to 150 and when she stood, it increased to 180 to 200. The electrocardiogram revealed paroxysmal auricular tachycardia. These changes invariably occurred whenever the patient stood.

Woofter and Deibert have also studied the association of orthostatic hypotension with tabes (142). One patient had a tachycardia even when recumbent. In addition to epigastric distress, abdominal cramps, lightning pains, Argyll Robertson's pupils, weakness in the legs and unsteadiness of gait, this patient had an absent libido and potentia, difficulty in urination, and hypoactive sweating. Fifteen additional patients with tabes did not have a decrease in systolic pressure greater than 12 mm. Hg on standing. An example of a patient with tabes dorsalis and orthostatic hypotension is the following:

J. J. was a 54 year old merchant seaman who had gonorrhea at age 18 and entered the Baltimore U. S. Public Health Service Hospital for the first time 22 years ago. At that time, he complained of weakness and numbness of the feet and legs. He had clinical and spinal fluid changes consistent with the diagnosis of tabes dorsalis. He was treated with bismuth and neosalvarsan injections. He was not seen again until 13 years later when he was found to have Charcot disease of the left knee joint. He complained of lightning pains. On examination, he was found to have Argyll-Robertson's pupils. In addition to joint lesions, he had absent deep tendon reflexes in the arms and legs, absent cremasteric and abdominal reflexes, positive Romberg test, diminished vibratory sense over the legs, and loss of proprioception in both feet. On many occasions he had orthostatic hypotension. His pressure fell from 140/95 mm. Hg in the supine position to 66/56 in the sitting position. His pulse rate did not increase concomitantly. A 500 ml. phlebotomy performed with the patient in the supine position resulted in a fall in pressure from 130/90 to 60/?. The pulse rate was stable at 80. Subsequent study during which the feet were elevated resulted in an increase in arterial pressure to a level of 170/105. The administration of 0.2 gm. of sodium amytal at hourly intervals for three hours was associated with a fall in pressure from 150/110 to 90/70. Atropine administration (1.0 mg. IV) did not alter the pulse rate. Sublingual administration of 0.6 mg. nitroglycerine resulted in a fall in pressure from 148/100 to 72/58. Administration of 0.3 cc. of norepinephrine subcutaneously resulted in a rise in pressure to 174/104. On one occasion, ether anesthesia resulted in a fall in pressure to 86/62. The pulse rate was not increased by exercise. The arterial pressure did not change on carotid sinus message. Electrical skin resistance studies indicated marked impairment of sweat gland function.

Occasionally syringomyelia and hematomyelia are associated with orthostatic hypotension (37). In addition, orthostatic hypotension is commonly seen following acute transection of the spinal cord (31, 75). Persistent orthostatic hypotension was found in an elderly woman with multiple cranial nerve lesions caused by a large brain tumor in the floor of the fourth ventricle.

The association of Parkinsonism and orthostatic hypotension is more than coincidental. Some patients with orthostatic hypotension have a stooped, shuffling gait, a coarse tremor and a mask-like facies. Crampton found nine patients with paralysis agitans in whom the diastolic pressure did not rise in the upright position (26). Other authors have reported Parkinsonism in cases of orthostatic hypotension (8, 84, 95, 102). Whether lesions of the corpora striata and optic thalami are responsible for certain types of orthostatic hypotension is conjectural. The basal ganglia may be of importance in postural regulation, but are not essential. Ingram and Ranson have shown that acute and chronic lesions of the red nuclei in cats do not interfere with righting reflexes, standing or walking (73). The postural effects of surgical chordotomy have been discussed by Johnson *et al.* (75) in their study of orthostatic hypotension following chordotomy.

In certain patients, central nervous system disease of unclassifiable type is present. The following is such a case.

G. D. (NIH #015848) was a 56 year old white man. At age 50 he had a decrease in libido and potentia. Two years later he had symptoms of nocturia, urinary hesitancy, and dribbling. He was found to have a neurogenic bladder and decreased anal sphincter tone. Over the next three years (1952–55) progressive lightheadedness and weakness in the neck, arms and legs developed. He developed bilateral exophthalmos not previously present and atrophy of the iris.

When seen in January, 1956, the patient complained of progressive muscular weakness, more marked in the mornings. There was increasing constipation and anorexia. He had developed dysarthria. At that time, arterial pressure was 145–150/90–95 mm. Hg. On standing, his pressure fell to 60/50 and his pulse rate rose from 92 to 110. He had pronounced dysarthria, bilateral abnormalities of adduction of the eyes, cerebellar ataxia and protrusion of the tongue to the left. There was no peripheral neuritis. Laboratory studies of significance were hematocrit 38, intermittent two plus albuminuria; NPN 44 mg. per cent; blood sugar 104 mg. per cent; B.M.R. minus 16; radioiodine uptake normal; PBI. 6.6 micrograms per cent, spinal fluid protein 10 mg. per cent, blood and C.S.F. serology negative, treponema pallidum agglutination and immobilization tests negative in blood and spinal fluid. Muscle biopsy was normal. Electrical skin resistance studies revealed a sweating defect below the tenth thoracic dermatome. There was free acid in the stomach. Neostigimine test was negative. Glucose tolerance test was normal. A myelogram was normal. A pneumoencephalogram revealed diffuse convolutional atrophy. The patient was admitted to the National Institutes of Health for the first time in January, 1957. His gait and speech had become progressively worse and he had noted irregular jerking of the right leg. He had difficulty in carrying out fine movements with his hands. There was a gross tremor of the arms and chin at a frequency of about 2 per second. Marked orthostatic hypotension without an associated increase in pulse rate was noted. A relaxed anal sphincter, ten degrees of exophoria, bilateral medial rectus paralysis, bilateral iris atrophy, questionable exophthalmos, and anisocoria were present. Tongue deviated slightly to the right with marked dysarthria. Abdominal and cremasteric reflexes were absent. The deep tendon reflexes were hypoactive. There was complete anhidrosis. All coordinated movements were slow. He had a mask-like facies, tremor of the upper extremities, and ataxia with a positive Romberg. On standing,

he had a marked kyphotic posture. The only sweating that occurred on heating at a temperature of 60°C for 30 minutes was in the axillae and on the upper lip. Laboratory tests showed: maximum and minimum urine concentrations of 1.026 and 1.009, one plus albuminuria, hematocrit 44 per cent and blood urea nitrogen 18 mg. per cent. Congo red test was normal. Plasma and urinary 17-OH corticoid determinations were normal. Glucose tolerance test revealed the following:

Time	*FBS*	*Urine*
Control	79	0
30′	139	Trace
60′	177	Trace
120′	187	2+
180′	113	1+

Full scale intelligence test was 122, classified superior. Quadriceps muscle biopsy revealed only atrophic muscle fibers with increased number of peripheral and central nuclei.

Autonomic neuropathic orthostatic hypotension

This type of orthostatic hypotension characteristically occurs in elderly men and is both persistent and severe. Although the sympathetic nervous system is clearly involved, all features of the syndrome cannot be explained by a lesion of the sympathetic nervous system alone. Abnormalities of the parasympathetic nervous system are suggested by: (1) the failure of atropine to alter the pulse rate; (2) the absence of bradycardia during administration of norepinephrine and neostigmine; (3) the lack of effect on the pulse rate and arterial pressure of carotid sinus massage; (4) the inability to have erections; and (5) the failure of hypoglycemia to produce an increase in gastric acidity.

H. C. (J.H.H. #426575, NIH #011606) was a 55 year old white man whose illness began ten years prior to study. After suffering for one year from syncopal attacks, he was discovered to have severe orthostatic hypotension. Early in his illness, he had profuse, hyperactive sweating over the trunk with absent sweating over the arms and legs. Several months after the initial symptoms, he developed partial, then complete impotence. Initially he had a tachycardia when his arterial pressure fell, but as the sweating defect increased, his cardiac accelerator nerves became involved and his heart rate became constant. On examination, he was robust and completely normal except that, on standing, his arterial pressure became unobtainable. He looked dazed. His legs and genitalia became cyanotic. He developed clonic movements of the arms, then fainted. No change in pulse rate was noted despite the fall in pressure. The pulse pressure diminished as the mean pressure fell. Hemogram and urinalysis were normal. A marked sweating defect was demonstrated by inspection, confirmed by electrical skin resistance and starch-iodine studies. The disease has been slowly progressive over a ten year period although the patient has been symptomatically improved by the injection of intramuscular vasopressin and by nasal inhalation of whole posterior pituitary powder. Underlying diseases known to be associated with orthostatic hypotension were excluded. Physiologic studies in this patient have been reported elsewhere (134).

L. S. (NIH #012785) was a 21 year old college student. Twenty-seven months prior to admission, he developed generalized weakness, anorexia and diarrhea. On standing, he com-

plained of dizziness and blurring of vision. He also noted a marked watery nasal and postnasal discharge associated with nasal stuffiness. There was occasional excessive lacrimation and salivation. Pain in the region of the parotid and submaxillary salivary glands occurred when he ate. For several months he was bedridden with postural hypotension. This was so severe that he had difficulty sitting up in bed.

In the first month of illness, he had difficulty in micturition and defecation. Early in his illness, he was found to have an elevated spinal fluid protein (99 mg. per cent) which gradually fell to normal in two months. There were no other spinal fluid abnormalities. Of possible significance is the fact that a slightly papular, erythematous, pruritic rash appeared over his trunk during the first month of his illness. Upon arrival of summer, five months after the onset of his illness, he noted that he no longer sweated. This was in marked contrast to the increased sweat gland activity that he noted at the beginning of his illness. Impotence and impaired ejaculations were also noted.

After the first year of illness, the diarrhea, anorexia and postural symptoms improved somewhat. At the time of admission to the National Institutes of Health, 27 months after the onset of symptoms, he complained of chronic diarrhea, extreme generalized weakness, blurring of vision, and syncope on standing. Positive physical findings were: emaciation, slight pectus excavatum, and orthostatic hypotension. General and neurological examination were otherwise normal. Urinalysis and hemogram were within normal limits. Spinal fluid examinations were normal, as were urinary 17-ketosteroid and plasma hydrocortisone levels.

After extensive study, the patient was treated with 2-methyl 9 alpha fluoro-hydrocortisone, a potent sodium-retaining adrenal steroid (90). During the past three years, he had done exceedingly well. He has returned to college. Despite the persistent orthostatic hypotension, diminished sweating and impotence, he has been able to have an almost normal life. He has continued to take the steroid, and is carefully observed for potassium depletion and excessive hypertension during recumbency.

LOCALIZATION OF THE LESION

Analysis of the pathogenesis of orthostatic hypotension must rest on a knowledge of how vasomotor regulation functions normally. It has been shown that many physiologic control mechanisms maintain and adjust arterial pressure under different conditions of activity and position. Accordingly, disease states occur in which one or several of the mechanisms for arterial pressure regulation are abnormal. In certain patients, the blood vessels themselves are unable to react normally, either permanently as in amyloidosis, or temporarily, as in adrenal insufficiency (34). In others, muscle wasting and deficient muscle tone results in impaired venous return. In still others, varicose veins or venous angiomata (25, 32) result in abnormal pooling of blood. In the majority of patients who have severe and persistent orthostatic hypotension, the defect seems to be in the nervous system. Functionally the vasomotor center may be divided into cardiac accelerator and vasoconstrictor components. Impulses arising in the peripheral vasosensitive areas inhibit these centers and maintain circulatory homeostasis through a dampening effect. Heymans has shown that cardiac slowing and vasodilatation begin when arterial pressure is about 60 mm. Hg and progress steadily as the pressure rises. A decrease in pressure results in

a progressive decrease in the moderating impulses arriving at the vasoconstrictor center along the glossopharyngeal and vagal depressor nerves. These stabilizing mechanisms are sensitive to an increase or decrease in pressure of as slight as 1 mm. Hg. It is unlikely, however, that pressure *per se* is monitored, but rather likely that the stimulus is distension of baroreceptors in the vessels. Section of these afferent buffer nerves in dogs results in severe hypertension and tachycardia.

Mild orthostatic hypotension with increase in pulse rate has been recorded in two patients in whom bilateral carotid sinus denervation and cervicodorsal sympathectomy were performed in the treatment of epilepsy (22). It is most unlikely, however, that abnormalities of the carotid sinus play a great role in patients with idiopathic orthostatic hypotension, since orthostatic hypotension has not been reported in other patients with denervated carotid sinuses. Furthermore, carotid sinus hypersensitivity results in a rather distinct clinical syndrome described by Weiss and Baker (141) and different from orthostatic hypotension.

Indirect evidence exists that the afferent fibers of the glossopharyngeal and/or vagal depressor nerves are intact in patients with orthostatic hypotension. The administration of dimethyl-phenylpiperazinium, a potent carotid body stimulant, to patients with idiopathic orthostatic hypotension results in marked hyperpnea (132). Furthermore, some patients hyperventilate when their arterial pressure falls. These are examples of afferent glossopharyngeal and depressor nerve function.

Since the afferent fibers are intact, the vasomotor center itself could be a possible site of the lesion. Some patients with orthostatic hypotension do not demonstrate the tachycardia that normally results from hypoxia (132). Despite the fact that hypoxia is thought to affect the medullary vasomotor center directly, its action is mediated through the efferent cardiac accelerator nerves which are frequently involved in autonomic neuropathy.

The hypothalamus has also been implicated as the site of the lesion in these patients. However, even when all centers above a spinal level are removed, both in the experimental animal (7, 66) or in human quadriplegic patients, vasomotor regulation returns to a considerable degree. Four of six patients with chronic quadriplegia resulting from cervical trauma at a C-4 C-6 level had no evidence of orthostatic hypotension when passively tilted to an upright position (135). This indicates that spinal vasoconstrictor mechanisms, normally subordinate to higher centers, are capable of independent activity. In most patients with autonomic neuropathic orthostatic hypotension, however, there is no return of vasoregulator mechanisms. On the other hand, spinal vasomotor reflexes, carotid sinus and aortic vasomotor reflexes, and the reflex hypertension induced by stimulation of the central end of the cut vagus nerve—all are abol-

ished by total peripheral sympathectomy (5, 66). In an analogous manner, sweating returns to the spinal man after recovery from spinal shock (119), but is usually persistently deficient in these patients. Similarly, reflex erection may occur in spinal man, but complete impotence is common in these patients. Thus, there is considerable evidence that this is a disease of the peripheral efferent autonomic nervous system. In diabetic neuropathy, the lesion is probably vascular affecting predominantly the non-myelinated fibers of the autonomic nerves (139). The syndrome of autonomic neuropathy with orthostatic hypotension can be closely mimicked both by extensive paravertebral sympathectomy and by chemical ganglionic blockade.

One cannot, however, explain all the defects on the basis of a peripheral sympathetic defect alone. Abnormalities of the parasympathetic nervous system are indicated by: 1) the failure of atropine to alter the pulse rate; 2) the absence of bradycardia during the administration of norepinephrine; 3) the lack of effect on the pulse rate and arterial pressure of carotid sinus message; 4) the inability to have erections; and 5) the failure of hypoglycemia to produce an increase in gastric acidity.

It is therefore concluded that the lesion in patients with idiopathic orthostatic hypotension is in the autonomic nervous system, involving both the sympathetic and parasympathetic divisions, and is probably distal to the medulla, in the spinal cord or beyond.

DIFFERENTIAL DIAGNOSIS

Usually the presenting symptoms and signs are referable to one of the following:

Diminished cerebral blood flow

Symptoms of dizziness, lightheadedness, and blurring of vision occur. The relationship to the sitting or standing position will often be noted. As cerebral anoxia progresses, weakness of the legs and buckling of the knees may occur. Jerking movements of the extremities progressing to a generalized convulsion may occur. Occasionally hearing as well as vision is impaired.

Generalized weakness

Although generalized weakness is characteristic of the hypotensive episodes, it may also be seen in the patients when recumbent. Characteristically, it is worse in the morning and disappears as the day progresses. In most patients, even when kept recumbent, one notes a gradual increase in the arterial pressure for several hours after awakening. Diurnal variations in strength and sense of well-being are also observed in hypertensive patients receiving ganglionic blocking agents and following extensive paravertebral sympathectomy.

Associated autonomic defects

Many patients complain of intolerance to heat. They may state that they do not sweat normally or perhaps not at all. Others describe a steady progression of impaired sweating, usually beginning in the lower extremities with sparing of the face and trunk. In others, the sweating defect may be severe and complete initially. A phase of hyperactive sweating may occur early in the course of the disease. This was observed in one patient who subsequently lost nearly all sweat gland function.

Impotence is a common finding, but seldom is the presenting complaint. Disturbances in micturition, diarrhea, constipation or alternating diarrhea and constipation—usually of long duration—occur in a small number of cases. Anorexia and weight loss are occasionally seen. Rarely excessive lacrimation, excessive salivation, rhinorrhea, and peculiar choking sensations in the throat occur.

These signs and symptoms suggest that the patient may have an autonomic dysfunction. Occasionally however, the patients will present in odd ways. One patient presented with the diagnosis of epilepsy. One was diagnosed after he had become hypotensive when anesthetized. These patients do not compensate for the vasodepressor effect of pentothal, ether and other anesthetic agents, and hypotension results. At the outset several patients were diagnosed as psychoneurotic.

If one suspects or wishes to rule out orthostatic hypotension, it is usually best to study the patient on a fluoroscopic table. The patient is secured lightly by an abdominal strap and can be passively tilted to any position. If severe symptoms result, he can be placed promptly and efficiently back in a recumbent position. In patients with orthostatic hypotension, graded tilting from the horizontal position results in an immediate and progressive fall in both systolic and diastolic pressure, directly related to the degree of tilt. As the degree of tilt increases, the patient may note progressive weakness, tired feelings, lightheadedness, weakness of the legs with possibly buckling of the knees, blurring of vision, deafness, drowsiness and loss of consciousness. Cyanosis and turgor of dependent parts, yawning, hyperpnea (rarely), clonic movements, and syncope are observed if the hypotension is severe.

It is important to note whether the patient complains of nausea, abdominal cramping or develops a cold, clammy sweat. These symptoms are seen in the so-called "vasovagal syncope" and must be differentiated from orthostatic hypotension. In vasovagal syncope, the diastolic pressure rises initially in contrast to the fall of diastolic pressure in orthostatic hypotension. After 5 to 10 minutes, the heart rate slows markedly, the pressure falls and a cold, clammy sweat appears (89). The patient may then faint. It is not known why patients with orthostatic hypotension do not develop the greenish pallor, nausea, ab-

dominal cramps and cold sweat seen in vasovagal syncope. Their absence in autonomic neuropathies suggests that they are of reflex origin.

With the patient on a fluoroscopic or tilt-table, one can carry out additional studies to determine the diagnosis. The pulse rate is very significant. If the pulse rate increases, the cardiac accelerator nerves are intact. If the pulse rate remains fixed, this is suggestive evidence of autonomic nervous system dysfunction. However, it must be emphasized that a fixed heart rate is not a *sine qua non* of the diagnosis of autonomic neuropathy.

If the patient has orthostatic hypotension, following the fall in arterial pressure associated with passive tilting, a characteristic "overshoot" of the pressure to levels higher than the baseline occurs after the patient resumes a recumbent position. In addition, if one performs the Flack test (42), a Valsalva maneuver performed by blowing against a standard pressure for a measured period of time, an exaggerated fall in arterial pressure occurs. The normal "overshoot" of the arterial pressure to levels higher than the baseline takes place immediately after the Valsalva maneuver (111).

The demonstration of supersensitivity to pressor agents, i.e., a marked rise in arterial pressure following the intravenous infusion of 2 micrograms of norepinephrine per minute, gives evidence that the patient does not have orthostatic hypotension on the basis of amyloidosis, adrenal insufficiency or potassium depletion. In these diseases, the vasculature has diminished rather than exaggerated reactivity.

Since hyperpigmentation of the skin and mucous membranes is not a feature of autonomic neuropathic orthostatic hypotension, its presence suggests adrenal insufficiency. In performing a commonly used test for adrenal cortical insufficiency, studying the patient's ability to excrete water, one must be aware that patients with autonomic neuropathy can excrete water normally only if kept recumbent during the test. This is illustrated in Figure 8. When one liter of water was given intravenously, urine flow increased, urine osmolality fell and the water was promptly excreted. This occurred when the patient was recumbent, arterial pressure and glomerular filtration rate (G.F.R.) being normal. In the second study when the patient was tilted upright as indicated in the figure by the fall in arterial pressure, the administration of one liter of water did not increase urine flow or decrease urine osmolality until the patient was tilted back to recumbency and the arterial pressure returned to normal. The increase in sodium excretion in the recumbent position was associated with elevation of the G.F.R. resulting from the initial expansion of the plasma volume with water.

In autonomic neuropathic orthostatic hypotension, plasma hydrocortisone levels and the response to ACTH are normal, ruling out Addison's disease.

The presence of peripheral neuropathy, Argyll Robertson's pupils, lightning

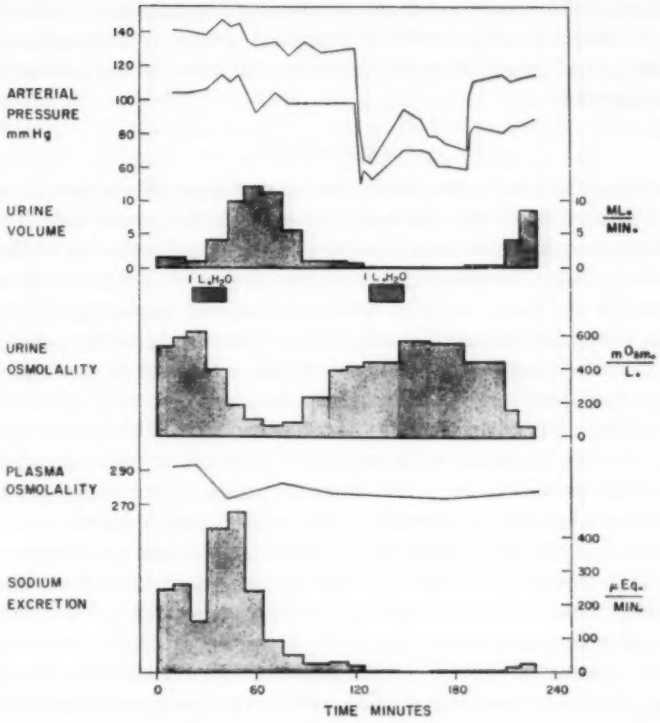

FIGURE 8. Arterial pressure, urine flow, urine osmolality, and sodium excretion in a patient with orthostatic hypotension. The fall in arterial pressure resulted from assuming an upright position. One liter of 5 per cent glucose was infused during recumbency and then again when the patient was upright. In contrast to the prompt excretion of the water when the patient was recumbent, he failed to do so when upright. The retained water was excreted when he became recumbent again as indicated by the rise in arterial pressure. The rise in sodium excretion was associated with a rise in glomerular filtration rate.

pains, or evidence of posterior column disease leads to the diagnosis of tabes dorsalis. These same signs and symptoms occur in patients with diabetic neuropathy however. Occasionally, patients with Parkinsonism have orthostatic hypotension. Other patients will have vague neuromuscular defects that cannot be classified as a definite neurological disorder.

Sweat gland studies, using the electrical skin resistance or other quantitative techniques, are useful in confirming autonomic neuropathy and provide a means of following the progression of the disease.

Acute hypovolemia, anemia, recent febrile illness, muscular wasting, and

generalized debility must be considered in the differential diagnosis. These are usually evident and do not result in persistent, severe and chronic orthostatic hypotension, but rather disappear with improvement in the patient's underlying disease state.

MANAGEMENT

Since there are specific treatments for several types of orthostatic hypotension, it is imperative to attempt to diagnose the underlying disease, if possible. Correction of acute hypovolemia, potassium repletion, replacement therapy in adrenal or pituitary insufficiency, careful regulation of diabetes mellitus, penicillin therapy for tabes, surgical removal of venous angiomata (74)—all are examples of specific therapy. In other cases, such as the orthostatic hypotension of convalescence, or that following acute paraplegia or quadriplegia, the vasoregulatory mechanisms will usually return to normal or near-normal activity after a period of time. In the quadriplegic patients this may take months, but the prognosis with respect to orthostatic hypotension is good. Progressively increasing the period of sitting and standing is possibly of value in hastening the return of circulatory homeostatic mechanisms.

Treatment of chronic, persistent and severe orthostatic hypotension may be divided into three aspects: (a) mechanical vasoconstrictors, (b) plasma and extracellular fluid volume expanders, and (c) pressor drugs. Several investigators have shown that immersion of the body in water up to the level of the heart will prevent the fall in arterial pressure (127). These experiments illustrate the effect of externally applied hydrostatic pressure in preventing the venous distension that results from the hydrostatic pressure exerted by the column of blood below the heart level. From the standpoint of long term management, attempts have been made to use elastic stockings and abdominal binders (1, 120). Recent advances in the development of anti-gravity suits have allowed fabrication of an elastic suit that can apply effective pressure without being inordinately restrictive. In ten patients with severe orthostatic hypotension (121), Sieker and his associates tried two different types of garments designed to apply counter pressure over the body from the waist down. An air-filled suit designed for flyers was generally more effective than an elastic garment. Four of ten patients were able to return to part-time employment. Several patients in whom we have used an elastic anti-gravity suit found them unsatisfactory, especially in the summer. In the absence of normal sweating a considerable part of their thermoregulation is due to peripheral vasodilatation. The suits inhibit this heat loss and aggravate the heat intolerance.

As has been previously shown, an increase in blood or extracellular fluid volume by the infusion of whole blood, albumin, or saline results in a marked elevation of arterial pressure and diminution of the symptoms of orthostatic

hypotension. The temporary improvement resulting from the infusion of two liters of normal saline has been found useful when these patients are to be operated upon, wish to travel from one place to another, or during radiologic examinations in a standing position. More prolonged hypervolemia may be achieved by the administration of adrenal sodium-retaining steroids and salt (9, 92, 124). Furthermore, the effect of sodium-retaining steroids may also be beneficial through a direct effect on the blood vessels (142). As previously mentioned, aldosterone release may be impaired in patients with orthostatic hypotension. The use of sodium-retaining steroids demands careful management by the physician, since hypokalemia and excessive hypertension in the recumbent position may be encountered.

Vasoconstrictor drugs are useful in the autonomic neuropathic group in whom a supersensitivity to vasoactive drugs can be demonstrated (2, 17, 18, 30, 35, 37, 46, 48, 54, 80, 81, 84, 86). These drugs are uniformly ineffective in those conditions in which the responsiveness of the vascular smooth muscle is impaired, i.e., adrenal insufficiency, amyloidosis, potassium depletion. In the other cases, ephedrine, paredrine, adrenaline, Aramine® and many other sympathomimetic drugs have been used, with varying degrees of success. The most commonly used drug is ephedrine, the usual dose being about 50 mg. by mouth on a regular schedule as frequently as necessary. Another useful pressor drug is vasopressin, given by intramuscular injection, or by nasal inhalation of whole posterior pituitary powder (21, 134). The latter is occasionally complicated by the development of excessive nasal irritation, presumably related to the vasoconstriction. In addition to the direct vasoconstricting effect of vasopressin, the expansion of the body water is helpful, although one must be wary of the development of water intoxication or excessive coronary vasoconstriction.

None of these therapeutic measures cures the patients. They are helpful, however, in enabling the patient to lead a more satisfactory life. In two patients with primary autonomic neuropathy, considerable spontaneous improvement was noted over a period of years. Both patients were in their twenties and, although the disease was still clearly present, they were able to lead nearly normal lives. In most patients, the disease is slowly progressive over a period of many years.

SUMMARY

(1) The *immediate* physiologic consequences of a change in the body's position from a supine to an erect position are:

(a) Dilatation of the vascular beds below the heart as a result of the increased intraluminal hydrostatic pressure.

(b) Decreased venous return to the right atrium.

(c) Decreased cardiac output.
(d) Decreased total peripheral vascular resistance.
(e) Decreased arterial pressure.
(f) Decreased cerebral blood flow.

(2) The most important *compensatory* mechanisms that preserve the constancy of the cerebral blood flow in normal persons and neutralize the physiologic disturbance of assuming the upright posture are:

(a) Decreased cerebral vascular resistance.
(b) Reflex arteriolar constriction with consequent increase in peripheral vascular resistance.
(c) Reflex venoconstriction with consequent decreased capacity of the veins and increased venous return to the right atrium.
(d) Increased tissue pressure in the legs, a pumping action of the leg muscles forcing blood past venous valves, reflex hyperventilation, and increased tension in the abdominal musculature—all of which effect an increased venous return to the right atrium.
(e) Reflex increase in heart rate with consequent increased cardiac output.
(f) Release of norepinephrine, antidiuretic hormone, and possibly aldosterone, effecting both increased vascular resistance and hypervolemia.

(3) Under certain conditions, the normally operating compensatory mechanisms are inadequate to maintain circulatory homeostasis:

(a) Gravitational stress, resulting from excessive gravitational forces.
(b) Thermoregulatory stress, resulting from fever or high environmental temperature.
(c) Exertional stress, resulting from muscular activity.

(4) In certain disease states the stress is not extraordinary, but the compensatory reactions are absent or deficient:

(a) Endocrinopathic orthostatic hypotension.
    1. Adrenal insufficiency.
    2. Panhypopituitarism.
    3. Hypothyroidism.
    4. Diabetes mellitus.
(b) Potassium depletion.
(c) Amyloidosis.
(d) Hypovolemia and/or anemia.
(e) Central neuropathic orthostatic hypotension.
    1. Tabes dorsalis.
    2. Syringomyelia.
    3. Hematomyelia.
    4. Spinal cord transection.
    5. Parkinsonism.

6. Unclassifiable central nervous system disease.

(f) Autonomic neuropathic orthostatic hypotension.

1. Post-sympathectomy.

(A) Surgical.

(B) Pharmacological.

2. Primary autonomic neuropathy.

(5) In categories (a), (b), and (c), the responsiveness of the vascular smooth muscle to norepinephrine, vasopressin, and possibly other vasoactive substances is abnormal. In both the central and autonomic neuropathies, reflex compensatory mechanisms are deficient.

(6) The history of the phenomenon of orthostatic hypotension, its differential diagnosis and the clinical management of the patients are discussed.

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A PRELIMINARY REPORT ON AN ANTIMYCOBACTERIAL SERUM TEST[1]

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The chemotherapy of tuberculosis as currently employed involves the use of multiple drugs, nearly always in combinations and at varying dosage levels. A patient with tuberculosis presents special problems related to bacterial metabolism, the reaction of the host to the infection, and response to antituberculous agents. These are some of the factors which make it difficult to outline a program of chemotherapy which can be followed for long periods with confidence. The purpose of the present report is to record some preliminary observations on a test which gives a rough estimate of the effect of a patient's serum on the specific mycobacterium, at different times during a day in which drugs are being given and at various intervals thereafter during the course of treatment.

Careful studies have been carried out by many investigators on the absorption and metabolism of the various antituberculous drugs. Interesting observations have been made on metabolic peculiarities of the salts of paraaminosalicylic acid (1). Other studies have demonstrated the fact that the antituberculous activity of isoniazid is limited almost entirely to the free as opposed to the acetylated form, changes which vary from one individual to another, but are remarkably constant for any one patient. It has likewise been shown that the serum level of active isoniazid may be raised by the simultaneous administration of paraaminosalicylic acid, a phenomenon explained by some workers as a competition between these chemicals for acetylation (2 to 7). Chemical tests are available for the quantitation of PAS in the blood and urine and the same holds true for isoniazid and its products in the urine, but methods for the determination of blood levels of free isoniazid have only recently been developed in a few laboratories. In the absence of a chemical method a bioassay for active isoniazid is being employed in several clinics and has been standardized as well as can be done with such a technique (8 to 11). Another method of considerable promise is one in which isoniazid is labelled with C^{14} and levels of the free drug estimated by tracing its radioactivity in serum and in urine (12). The preliminary phases of this study have been carried out on rats, but the investigators have found the test to be equally valuable in humans. If the therapeutic regimen is guided by such tests, one may obtain a satisfactory

[1] Read in part at the meeting of the Eastern Section of the American Trudeau Society in Boston October 31, 1958.

clinical response in the great majority of cases, by using the combination of PAS and isoniazid that is best suited to the individual patient (11).

The problem is not always as easily managed as this, however, because many complicated cases of tuberculosis are still encountered. The complicating features may be related to drug resistance, to the persistence of cavities or to the simultaneous presence of another disease. Many patients who come in for surgery receive multiple drugs in hopes that they are adequately prepared. It is difficult to predict the outcome of such cases when we know so little about the serum levels of more than one or two of these drugs. The tendency to treat cases of this sort with multiple antituberculous agents suggests the need of a test to measure the effective level of a combination of drugs in the patient's serum. A procedure of this type would have additional meaning if the specific mycobacterium were employed as the test organism. It is impossible to tell—on the basis of sensitivity studies alone—whether or not a combination of drugs will be synergistic, additive or even antagonistic in their effect.

The test described in this report has been developed in an attempt to provide an answer to some of the questions mentioned above. The general principle has been to obtain a sample of blood serum before treatment has been started and then to make several observations on the same patient at various intervals after the medicines have been given. In any one patient, therefore, we would hope to obtain information regarding effective blood-levels as related to the length of time since the administration of the drugs. In a few patients with tuberculous meningitis simultaneous readings were made on serum and spinal fluids.

MATERIALS AND METHODS

The medium employed for growth of the tubercle bacilli is the liquid medium of Dubos and Middlebrook (13). It is prepared as follows:

KH_2PO_4	1.0 gm.
$NaHPO_4 \cdot 12H_2O$	6.3 gm.
Asparagin	2.0 gm.
Enzymatic digest of Casein	2.0 gm.
Dissolve in glass-distilled water, then add Ferric Ammon. Citrate	0.005 gm.
$MgSO_4 \cdot 7H_2O$	0.01 gm.
$CACl_2$	0.0005 gm.
$ZnSO_4$	0.0001 gm.
$CUSO_4$	0.0001 gm.
Tween-80	5 cc. of stock solution

Make up to 900 cc. with glass-distilled water[2]

The basic medium is autoclaved at 12 lbs. pressure for 15 minutes and allowed to cool. One hundred cc. of 5 per cent bovine albumin is then added. This clear solution is distributed aseptically into sterile screw-capped test tubes in 2 cc. amounts.

[2] We found it necessary to use glass-distilled water because any other method of distillation resulted in a large residue completely masking the growth of the tubercle bacilli.

TABLE I

Antimycobacterial Effect of Sera Compared With Clinical Findings

Name and No.	Diagnosis	General Condition	Drugs† (daily doses)	How‡ Given	Time Since Last Dose	Type of Specimens	Results*					Remarks
							4	8	16	32	64	
1) 339912	Pulm. tbc. (far adv.)	Poor	PAS 12	3 × 4		Serum	+	+	+	+	++	Weak inhibition in a patient making poor progress.
2) N.J. 658487	Pulm. tbc. Sarcoid	Very sick. Improving	Distrycin 2 INH 300	1 × 2 100 × 3		Serum	0	0	0	0	+	Good level. Correlates well.
3) W.P. 665813	Pulm. tbc.	Satisfactory	Stm 1 PAS 12	b.w. 3 × 4	3 days 5 hrs.	Serum I	0	0	0	0	0	Higher level 3 days after than 5 hrs. after streptomycin.
					5 hrs. 5 hrs.	Serum II	0	0	0	0	++++	
4) J.D. 532729	Pulm. tbc. (Resection)	Good	Stm 1 PAS 12	q.d. 3 × 4	1 day	Serum	0	0	0	0	+	High level of inhibition.
5) Carp. (Vets. Hosp.)	Pulm. tbc. (far. adv.)	Poor	Stm 1 INH 300	b.w. 1 × 3	1 day 2 hrs.	Serum	++++	++++	++++	++++	++++	No inhibition. Good correlation.
6) T.B. 678745	Pulm. tbc. (far adv.) (Gaffky X)	Very sick. Improving	Stm 1 Vio 3 PAS 12 INH 300	q.o.d. 1 × 3 3 × 4 100 × 3		Serum I	0	0	0	0	++++	Satisfactory levels. Last specimen 1 day after thoracotomy.
			Vio 3 INH 300	1 × 3 100 × 3	1.5 hrs. 1.5 hrs.	Serum II	0	0	0	0	++++	
7) R.C. 688168	Pulm. tbc. (far adv.) Bronch. Pleural fist.	Unsatisfactory	Vio 2 INH 300	0.5 × 4 100 × 3		Serum	0	0	++++	++++	++++	Low levels. Good correlation.
8) J.F. 223150	Pulm. tbc. Sarcoid	Satisfactory	Stm 1 INH 300	q.o.d. 100 × 3	4.5 hrs. 4.5 hrs.	Serum	0	0	0	++++	++++	Fair level. Responding well.
9) R.A. 665373	Pulm. tbc.	Good	Stm 2 PAS 16 INH 400	0.5 × 4 4 × 4 100 × 4		Serum	0	0	0	0	++++	Good level but no better than with smaller doses in other patients.
10) H.F.	Pulm. tbc.	Improving	Pen. 0.6			Serum	++++	++++	++++	++++		First specimen taken before

			INH 400	100 × 4								in other patients.
10) H.F. 724459	Pulm. tbc.	Improving	Pen. 0.6 m.			Serum I	++++	++++	++++	++++		First specimen taken before specific therapy.
			Achro-mycin 2									
Same 2 wks. later			Stm 1	b.w.	5 hrs.	Serum II	0	0	0	+++	++++	
			INH 300	100 × 3	9 hrs.							
11) T.M. 346617	Pulm. tbc.	Very sick. Improv-ing	Pen. 3 m.			Serum I	++++	++++	++++	++++		First specimen before therapy. No inhibition though patient was improving.
			Achro-mycin 1									
			INH 300	100 × 3		Serum II	++++	++++	++++	++++		
			PAS 12	3 × 4								
12) A.V. 725753	Pulm. tbc.	Satisfac-tory	Stm 1	q.d.	23 hrs.	Serum I	0	++	++++	++++		Difference of titers consistent with presumable drug levels in sera.
			PAS 12	3 × 4	1 hr.							
				q.d.	6 hrs.	Serum II	0	0	+	++++		
				3 × 4	4 hrs.							
13) M.Q. 774302	Pulm. tbc.	Very sick	None			Serum I	++++	++++	+++	++++		Inadequate level. Patient in poor condition.
			Stm 1	q.o.d.	4 hrs.	Serum II	0	+++	++++	++++		
			INH 300	150 × 2	10 hrs.							
			PAS 8	2 × 4	4 hrs.							
14) J.B. 231178	Pulm. tbc.	Fair	Stm 1	b.w.	2.5 days	Serum I	0	+++	++++	++++		Low titer in both tests, though patient showed some improvement.
			PAS 12	3 × 4	5 hrs.							
			Stm 1	b.w.	6 hrs.	Serum II	0	0	++++	++++		
			PAS 12	3 × 4	3 hrs.							

* Amount of growth noted at increasing dilutions of serum.

† INH refers to Isoniazid; doses are given in milligrams. The doses of all other drugs are given in grams.

‡ The first number refers to the single dose, the second to the frequency of administration, q.d. means once a day, q.o.d. every other day and b.w. twice a week

TABLE II

Results in Three Patients with Tuberculous Meningitis

Name, No. & Date	Diagnosis	General Condition	Drugs (daily doses)	How Given	Time Since Last Dose	Type of Specimens	Results					Remarks
							4	8	16	32	64	
a) L.J. 660673 1/4/54	Pulm. and meningeal tbc.	Fair	Stm 1	q.d.		Serum I	0	0	0	++	++++	Specimen taken 3 weeks apart with similar results. Spinal fluid only slightly lower than serum.
			Distrycin 2	1 × 2		C.S.F. I	0	0	+	++	++++	
			INH 200	100 × 2								
			Stm 100 mg.	i.th.								
1/26/54	Pulm. and meningeal tbc.		Distrycin 2	1 × 2		Serum II	0	0	0	++	++++	
			INH 400	100 × 4		C.S.F. II	0	0	+	++	++++	
			Stm 100 mg.	i.th.								
b) J.F. 687562 10/26/54	Pulm. and meningeal tbc.	Satisfactory	Distrycin 2	1 × 2		Serum	0	0	0	++	++++	Responding well. Adequate level in blood and spinal fluid.
			INH 400	100 × 4		C.S.F.	0	0	+	+++	++++	
			PAS 12	3 × 4								
			Stm 50 mg.	i.th.	4 days							
c) J.L. 766562 3/26/57	Meningeal tbc.	Improving	Stm 2	1 × 2	6 hrs.	Serum I	0	0	+++	++++	++++	
			INH 600	200 × 3	8 hrs.	C.S.F.I	0	0	+	++++	++++	
			PAS 12	3 × 4	2 hrs.							
			Meticorten 60									
4/10/57	Meningeal tbc.	Improving	Stm 2	1 × 2	6 hrs.	Serum II	0	0	0	0	++++	Note inhibition of growth in high dilutions in serum and spinal fluid. Correlated well with clinical condition.
			INH 600	200 × 3	8 hrs.	C.S.F. II	0	0	0	+	+++	
			PAS 12	3 × 4	2 hrs.							
			Meticorten 60									
4/23/57	Meningeal tbc.	Much improved	INH 600	200 × 3	30 min.	Serum III	0	0	0	0	++++	
			PAS 12	3 × 4	2 hrs.	C.S.F. III	0	0	0	+	++++	
			Distrycin 1	1 q.o.d.	30 hrs.							
			Meticorten 15	5 × 3	2 hrs.							

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The sera were collected as follows: 5 cc. of blood was removed from the patient aseptically and placed in a small sterile test tube. Following the formation of a clot the blood was centrifuged and the serum poured off. These specimens were kept in the frozen state until used. Whenever possible, a control serum was obtained before therapy had been started. Subsequent specimens were collected at appropriate intervals after the patient had been started on antituberculous drugs.

The primary isolation of the tubercle bacilli was obtained on Lowenstein and Jensen's or on A.T.S. medium, the media which are used routinely in the Tuberculosis Laboratory of the Johns Hopkins Hospital. A growth was usually obtained under these circumstances in 4 to 6 weeks. The culture was next ground in a Ten-Broeck glass grinder, and a heavy inoculum seeded into 2 cc. of freshly prepared Dubos medium. After 7 days incubation the cultures were shaken vigorously and allowed to stand at room temperature for ten minutes. One tenth cc. of this preparation was then added to test tubes containing the patient's serum, diluted serially in the same medium from 1:4 through 1:64. The total amount of fluid in each tube was 2 cc. After two weeks' incubation at 37°C. the growth of the tubercle bacilli was recorded as 0 to 4 plus on the basis of the gross appearance of the cultures. Controls were set up with each test. The Figure 0 signified no visible growth, whereas 4 plus designated growth equal to that in the control tube, to which no serum had been added. Gradations in inhibition of growth were indicated by the symbols 1 plus to 3 plus.

RESULTS

A total of 62 such assays were carried out on 30 patients. Included in the study were 3 cases of tuberculous meningitis. All the other patients had pulmonary tuberculosis, which was far advanced in the majority. Many of the tests were done on the latter group either before or after some form of chest surgery.

TABLE III

Multiple Tests on Two Additional Patients

Name and No.	Serum No.	Drugs	Dose	Hours since med.	Inhibitory level	Sensitivities
J.C. 465178	I	(Penicillin)	Control	5	0	Strep: sensitive to 100; resistant to 10 mcgm.
	II	INH	800 mg. (200 × 4)	5	1:4	INH: sensitive to 5 mcgm. Slightly resistant to 1 mcgm.
		PAS	12 gm. (4 × 3)	16		
	III	INH	800 mg. (200 × 4)	2	1:8	
		PAS	12 gm. (4 × 3)	3		
	IV	INH	800 mg. (200 × 4)	5	1:8	
		PAS	12 gm. (4 × 3)	3		
	V	INH	800 mg. (200 × 4)	2	1:16	
		PAS	12 gm. (4 × 3)	1		
E.G. 818614	I	INH	600 mg. (200 × 3)	4	1:8	Strep: sensitive to 10 mcgm.
		PAS	8 gm. (2 × 4)	4		INH: sensitive to 1 mcgm.
	II	INH	600 mg. (200 × 3)	2	1:32	
		PAS	8 gm. (2 × 4)	2		
	III	INH	600 mg. (200 × 3)	6	1:32	
		PAS	8 gm. (2 × 4)	6		

FIG. 1. Heavy mycobacterial growth in all except the 1st tube, where the serum dilution is 1:4.

FIG. 2. Inhibition of growth to a dilution of 1:32. Apparent cloudiness of 1st tube due to serum being present before incubation.

Control tube not shown in either figure.

A notation was made concerning the patient's general condition at the time of the test, in order to provide some clinical information for the purpose of comparison. These data represent rough approximations, based as they are on isolated observations and the opinions of the professional staff. The total daily amount of each drug was recorded, with the size of the individual doses and the method of administration. The length of time from the taking of the medicines to the testing of the blood is obviously of considerable interest, and exact figures were obtained whenever possible. All sera obtained from a single patient were tested at the same time so as to guarantee uniformity of technique. It was interesting to find that the readings could usually be designated 0 or 4 plus, the lines of demarcation between no visible growth and heavy growth being quite distinct in the majority of the tests.

An attempt was made to correlate the results of the tests with the patient's apparent response to therapy, interpretations which obviously depend on long-term follow-up rather than isolated observations. We were particularly interested in data concerning 3 patients with tuberculous meningitis, in whom simultaneous readings were obtained on blood and spinal fluid. In these cases growth-inhibitory activity was demonstrated in the spinal fluids at levels nearly identical with those of the sera. These findings seemed to be well correlated with the clinical picture in this small group.

The pertinent findings are summarized in Tables I, II and III, and illustrated by Figures 1 and 2.

DISCUSSION

Many more observations will be required before we can estimate the place of any test of this sort in the therapeutic armamentarium of tuberculosis. The majority of the sera taken from patients receiving antituberculous drugs produce significant mycobacterial inhibition. No such activity was demonstrated before treatment had been started. In some tests visible growth was just as abundant in the first as in the last tube—an indication that no effective blood level could be shown in this manner. Sometimes this was in line with other clinical and laboratory findings but in others the correlation was not so definite. The value of the procedure in cases of this type will be decided by careful evaluation of each patient during a long period of observation.

The procedures which are being used currently in other clinics were described earlier. They consist principally of sensitivity studies, chemical determinations and bioassays for isoniazid blood levels. Data acquired in this manner should provide sufficient collateral information for all except a minority of the patients in any large clinic. However, in some of the more complicated cases, those particularly who are receiving several drugs, information of the sort described in this report may prove useful from a therapeutic and prognostic standpoint. The chief function of the test would seem to be to supplement the other laboratory procedures and to relate all of these findings to the clinical condition and progress of the individual with tuberculosis.

SUMMARY

1. The purpose of the procedure described in this report was to estimate the inhibitory effect of the sera of a group of patients with tuberculosis on the growth of the specific strains of mycobacteria.
2. Several specimens of blood were obtained from each subject whenever possible. A specimen taken before the institution of drug therapy served as a control.

3. The control sera uniformly failed to show any antimycobacterial activity, whereas significant levels of inhibition were demonstrated in the majority of the cases during the period of treatment.
4. Three cases of tuberculous meningitis were included in the study. In each of these patients growth inhibitory levels in the spinal fluids were found to be comparable to those in the sera.
5. A test of this sort should prove most useful as an additional guide in the treatment of complicated cases of tuberculosis, particularly those who are receiving several drugs.

Addendum

A recent report by Kreis (14) is pertinent to the present studies. A technique is described by which the specific tubercle bacilli are smeared on slides, incubated in dilutions of the patient's blood—before and during treatment—then examined microscopically for the extent of bacterial multiplication. The results usually showed a good correlation with sensitivity tests and with patient's response to therapy.

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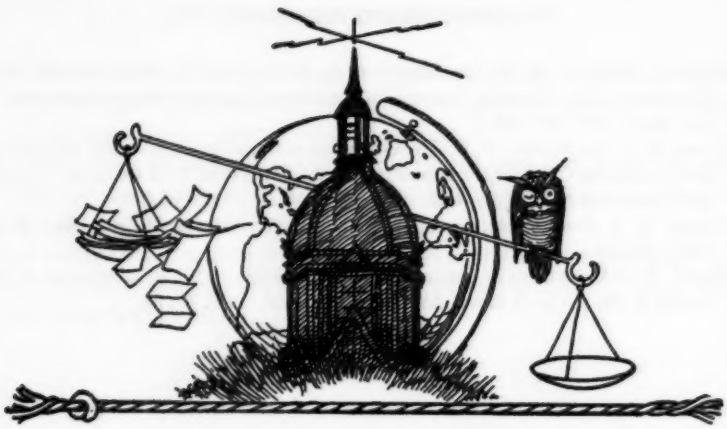

BOOK REVIEWS

J.-M. Charcot: His Life—His Work. By GEORGES GUILLAIN. Edited and Translated by PEARCE BAILEY. 202 pp., $7.00. Paul B. Hoeber, Medical Book Dept. of Harper & Bros., New York, N. Y.

A lamentable gap in medical biography has at long last been filled—and in more than merely adequate fashion. There has been, until now, no full length account of the life of the great French neurologist, Jean-Martin Charcot. Thanks to the graceful translation provided by Pearce Bailey, Director of the N.I.N.D.B., Georges Guillain's accurate and circumspect biography has been rendered into English. One is indeed tempted to call this an "official" biography. Guillain is a distinguished neurologist, the senior author of the Guillain-Barre syndrome. Too young to have known Charcot himself, he was a pupil of Raymond and Marie, two of Charcot's most illustrious disciples, and occupied, from 1925 to 1948, the very professorship in diseases of the nervous system that had been created for Charcot at the Faculty of Medicine in Paris. He has brought together official documents, memoirs, and personal conversation with those who made rounds with this founding father of neurology to present as complete a portrait as the evidence permits; he has disdained to embellish his account with romantic inventions of his own. All in all, he has created a volume of great interest to every student of the history of medicine—which is to say, to every physician with a pride in his heritage and a curiosity about its evolution.

Charcot, the son of an obscure Parisian carriage builder, rose to become the possessor of the world's first professorial chair in neurology, a discipline created in large part through his own efforts. Appointed Medecin de l'Hospice de la Salpetriere in 1862, he brought clinical order out of a chaos of chronic infirmities and, in the process, transformed what was at the same time an asylum, a prison, and a hospital into the leading center for clinical neurologic research. He established a series of neurologic entities, to which his name is still attached (the Charcot joint in tabes, the Charcot triad in multiple sclerosis and the Charcot-Marie-Tooth peroneal muscular atrophy will serve as instances). It was he who sent out as missionaries of the new gospel (though certainly they came to him as gifted pupils; greatness attracts the great) a luminous succession of disciples; consider only Raymond, Marie, Bourneville, Gilles de la Tourette, Babinski, Bechterew, Darkschewitsch, to mention but a few! By now, all know that it was at Charcot's clinic that Freud was first introduced to the mysteries of hysteria and hypnotism, an event that was to prove decisive

in the further history of psychiatry. But, perhaps more than any of these, it was his distinction to have developed the method of clinico-anatomic correlation which today remains the cornerstone of clinical neurology.

How shall we understand these prodigious accomplishments? Was there anything in the early life of the great man to foreshadow his future genius? If there was, it has been lost to history. What personal traits might account for it? We are told that he was a man of great energy, fixed purpose, and unswerving devotion to his conception of what was right, with the inevitable egocentricity this demands. He was well read, loyal to his associates, and charming as a host. How much did he owe to his predecessors? The Salpetriere had known Pinel, Esquirol, Baillarger, Falret and Magendie (among others). Surely, the divine spark had continued to glow among the embers of their work. And Charcot's was an era of the flowering of French science. But finally, we are left, as always in the face of greatness, with unknowns in the personal equation that we sum up as "genius," which serves, not as an explanation, but only as a restatement of our ignorance.

Professor Guillain goes at some length into the controversy over Charcot's views on hysteria and hypnotism, evidently much more of a live issue in France than here. In so doing, he becomes unnecessarily defensive of Charcot, who stands or fails, not on this, but on his more strictly neurological accomplishments. It would seem that the very analytic capacities that enabled Charcot to order neurologic disorders into discrete entities betrayed him in his efforts to deal with hysteria. Trying to force the multiform, plastic and field determined phenomenology of hysteria into categories and stages on the model of a neurologic disease, he ended by creating stereotypes among a colony of patients whose lives centered about the exhilarating experience of being presented at the grand spectacle of Charcot's clinics. To these public appearances, he gave meticulous attention; like many great teachers, he had histrionic talents of his own. His patients, undoubtedly awed by the presence of the master, performed as expected and Charcot saw no reason to doubt his own formulations. Moreover, it appears that in the later years, he did not personally examine patients but relied for this upon his assistants; one may suspect that his internes must have been determined that the chief not be disappointed. Remember, so much more sophisticated a psychological observer as Freud was misled by his hysteric patients into accepting the reality of early "memories" of sexual traumata, which only later was he to recognize as fantasies that served emotional needs. And he, too, found it necessary to abandon hypnosis as a technique in favor of the method of free association, an abdication that has only latterly been reexamined.

His ideas on hypnotism were inferior to those of Bernheim and Janet, his ideas on hysteria naive. He fought for his conceptions as he had defended his other theses. There is a hint, however, that at the very last he was preparing to reconsider his theory and "to dynamite the edifice to which he had personally contributed so much . . ." (Guinon). Be that as it may, it should also be said that his very interest in the problems of hysteria served to bring it to the fore as a legitimate medical disorder. His views on hypnotism are less readily explained away; his own stand was inadequate in the face of the insights of Bernheim whose correctness he failed to recognize. But no man is great in all respects and each is a creature of his times. In Freud's assessment: "Although scientific progress must change much of what we owe to Charcot, there is no question that the glory of Charcot will always stand above the opinions of his time."

A clue as to the single-mindedness and, at times, intemperateness, with which Charcot pursued his convictions is provided by the life of his son, Jean, who had set his heart upon a naval career. His father, however, would have none of it and Jean obediently entered medicine, ultimately becoming Chief of Clinic under Raymond at the Salpetriere. Shortly

after his father's death, he gave up medicine to begin a new career as a maritime explorer. He wrote at the time: "Should I succeed, that would be the best way to honor my father's memory, because the name of Charcot would have been honored twice." And this was to prove the case in the course of many polar voyages till his death at 69 during a violent gale off the coast of Iceland.

There remains only to comment that the reader will find this biography a rewarding excursion into medical history. Medicine being at least so much an art as it is a science, its history is peculiarly the history of its great practitioners. Dr. Guillain has placed us greatly in his debt by his labor of love; the definitive history of the relation of Charcot's concepts to the work of others remains to be written, but the first steps have been taken. Many thanks are due to Dr. Bailey for a translation, whose courtliness and reserve are in keeping with the author's purpose and for footnotes and a glossary that clarify references less familiar to an American than a French audience.

LEON EISENBERG

Synopsis of Ophthalmology. By WILLIAM H. HAVENER. 288 pp., $6.75. The C. V. Mosby Co., St. Louis, Mo.

This small primer outlines eye disease at a level not beyond the junior medical student. It is not sufficiently advanced to interest the ophthalmologist, and a well trained young physician, even in another specialty, will probably find much of the material beneath his level. However, it should be excellent for student nurses and interested laymen. The illustrations are profuse, but a significant proportion are of poor quality.

J. LAWTON SMITH

The Surgeon and the Child. By WILLIS J. POTTS. 225 pp., $7.50. W. B. Saunders Co., Philadelphia, Pa.

Willis Potts has written a charming book and obviously enjoyed himself doing it. He writes with engaging humor and refreshing candor and above all, beautiful simplicity and clarity.

The book will be easily read by laymen and yet it represents the relaxed evaluation of a great experience in pediatric surgery. The pediatric surgeon will find unequivocal statements of Dr. Potts' stand on all of the possible disputed points in each of the three or four dozen subjects discussed and the pediatrician will find lucid descriptions of surgical conditions, their recognition and treatment. For the student in the third year of medical school (year IV of the new curriculum) the book is ideal. Dr. Potts covers a great deal of ground easily and smoothly—if confessedly didactically and his prose reads like that of a light novel.

MARK M. RAVITCH

Synopsis of Ear, Nose and Throat Diseases. By ROBERT RYAN, WILLIAM THORNELL AND HANS VON LEDEN. 383 pp., $6.75. The C. V. Mosby Co., St. Louis, Mo.

This small handbook contains four major sections and discusses the applied anatomy, physiology, clinical pathology and treatment of diseases of the ear, nose and paranasal sinuses, pharynx and larynx.

All illustrations are in black and white and no radiographs are reproduced; however, the illustrations are adequate for a handbook. The authors assume that the specialist will have more extensive texts available for study and reference.

The book gives an excellent description of the common ear, nose and throat diseases. Treatment is outlined and agrees with current medical practice in this country.

In summary, this synopsis is an excellent fundamental handbook for medical students, interns, nurses, speech pathologists, and first-year residents in otolaryngology.

FRANCIS I. CATLIN

Speech and Brain-Mechanisms. By WILDER PENFIELD AND LAMAR ROBERTS. 286 pp., $6.00. Princeton University Press, Princeton, N. J.

Most discussions of disturbances of language are based upon psychological formulations with little, if any, reference to the neuronal mechanisms which may be involved. The authors of this small monograph base their considerations of speech upon the disturbances of cerebral activity which may be induced by the application of an electric current to localized areas of the cerebral cortex or the removal of small parts of the brain. Thus this approach gives a rather unique perspective to the problems of speech.

The material presented in the book formed the matrix for the Vanuxem Lecture given by Dr. Penfield at Princeton University in 1956. Because his audience included lay persons, medical students and physicians, the introductory chapters deal with the elementary aspects of the physiology of the cerebral cortex. The remainder of the book concerns the demonstration of speech alterations in the course of diagnostic and therapeutic procedures made before and during operation for the excision of epileptic scars. Dr. Penfield's wide clinical experience serves as a basis for the delineation of three cortical areas, one in the inferior frontal region, one in the anterior paracentral region and one in the posterior Sylvian region, which are closely related to speech. The authors believe that these have two-way connections to the thalamus which they suggest should also be an important station in speech mechanisms.

A number of interesting points are raised in this monograph. The relationship of cerebral dominance to language and its disturbances is thoroughly analysed. It is apparent that handedness is not necessarily directly related to cerebral dominance, but that other factors may play an important role. The occurrence of aphasia in bilingual individuals and polyglots is commented upon. Although patients have been reported to have had a speech disturbance in one language only, the authors have not seen such a dissociated aphasia. There has always been an affection of all languages, although one may be more disturbed than another. This would be in keeping with the modern concepts of the nature of language. In the concluding chapter the authors discuss some of the factors in the learning of foreign languages and present a strong plea for the teaching of such languages early in the educational system when children find it so simple to learn.

Although this monograph does not make any attempt to describe the psychodynamic processes in language or speech, it does emphasize the fact that there is a certain anatomical matrix upon which the speech and language faculties are based. In this way it presents much of interest and value concerning the cerebral mechanisms of speech which should be read by all who wish to study the higher mental functions.

A. EARL WALKER

The Mouth. Its Clinical Appraisal. By A. B. RIPPLE. 118 pp., $3.50. J. B. Lippincott Co., Philadelphia, Pa.

There is seldom an opportunity, such as this, for the medical and dental professions to have access to a publication that will act as a liaison for the two on the subject of the mouth. As Sir William Osler has said, "The mouth is oft-times the diagnostic mirror of the body." He also has implied that the mouth is the diagnostic "no-man's land".

Dr. Ripple, in the opening remarks of the preface states the problem full well: "A true

stomatologist would be both a physician and a dentist. He would possess a medical degree and would have had years of medical experience. In addition, he would hold a dental degree, with an equivalent foundation in the practice of dentistry. There are a few who have been graduated both from medical and dental schools by original intention, as dentist-turned physician, or less frequently, as a physician-turned dentist. The most of these practice oral surgery, not stomatology."

The book is written very concisely, and in a comprehensive and straightforward manner. It should serve very well as a handbook for orientation for the medical intern and clinician. For the dental intern and extern and practitioner, as a good review of the subject. The Rochester General Hospital has begun an innovation for a dental extern project, where recent graduates serve a morning a week for a year, to gain experience.

Chapter Four, in regard to anesthesia and drugs, might be considered to be a bit extraneous, but very interesting reading. For further editions of a book of this type, it might be suggested that color plates showing lesions of a benign nature and malignant lesions in an adjacent position be included. The fields of histopathology and microbiology are other subjects unto their own, and have wisely been deleted by the author as the title implies.

As Dr. Ripple concludes, "The physician who persists in learning will be rewarded on the day he finds his first malignant growth before it is too late, and on the day he detects foci of infection in his diabetic, his nephrotic, his rheumatic heart and his arthritic patients. These physicians and dentists will have justified the trust their patients place in them, and life will be better."

LOREN W. GRUBER

Bigger's Handbook of Bacteriology, 7th edition. By F. S. STEWART. 611 pp., $8.00. The Williams & Wilkins Co., Baltimore, Md.

In the preface to the first edition, written at Trinity College, Dublin, in 1924, Dr. Bigger stated that this handbook was intended as a short textbook, suitable for the use of medical students who commonly complain that, in the few months devoted to the study of bacteriology in the medical curriculum, they have not sufficient time to master lengthy textbooks. This seventh edition, which was prepared by Dr. Stewart, follows the aim of the original author in presenting in a relatively brief text "all the more important facts relating to bacteria as far as they affect man." It has been brought up to date by substantial additions to and rewriting of the original text, particularly in the fields of virology and chemotherapy.

If this handbook is judged in the light of the rather questionable purpose for which it is intended, it can be regarded as quite satisfactory. It contains a vast amount of information, clearly and concisely, if briefly, stated. The material is surprisingly complete for such a small text and has been brought well up to date. In contrast to some British texts, the nomenclature and methods are generally those in common usage in this country.

However, if this book is to be considered as a text in microbiology as taught in American schools of medicine, it must be judged inadequate. Important subjects are dismissed with a brief paragraph or even a single sentence and the effort to condense the material occasionally results in misleading or confusing statements. The most grievous fault is the complete absence of references and bibliographies.

In spite of these shortcomings, this handbook could possibly be as popular with American medical students as it has obviously been with those of the author's native Ireland, and may well be more satisfactory than some handbooks now in general use in this country. The cost of the volume, however, may mitigate its widespread acceptance as a study aid.

ISABELLE G. SCHAUB

BOOKS RECEIVED FOR REVIEW

The Arterial Wall. Aging, Structure, and Chemistry. Edited by ALBERT I. LANSING. Sponsored by The Gerontological Society. 259 pp., $7.50. The Williams & Wilkins Co., Baltimore, Md.

The Biology of Myelin. Vol. 4 of the Progress in Neurobiology Series. Edited by SAUL R. KOREY AND JOHN I. NURNBERGER. 405 pp., $9.50. Paul B. Hoeber, Inc. Medical Book Dept. of Harper & Bros., New York, N. Y.

Clinical Auscultation of the Heart, 2nd edition. By SAMUEL A. LEVINE AND W. PROCTOR HARVEY. 657 pp., $11.00. W. B. Saunders Co., Philadelphia, Pa.

Essential Principles of Pathology. By JOHN W. LANDELLS. 278 pp., $5.00. J. B. Lippincott Co., Philadelphia, Pa.

The History of Nursing. An Interpretation of the Social and Medical Factors Involved. By RICHARD H. SHRYOCK. 330 pp., $5.00. W. B. Saunders Co., Philadelphia, Pa.

The Life and Times of Sir Charles Hastings. By WILLIAM H. MCMENEMEY. 516 pp., $10.00. E. & S. Livingstone Ltd., Edinburgh and London. The Williams & Wilkins Co., exclusive U. S. agents, Baltimore, Md.

Pediatric Pathology. By DANIEL STOWENS. 676 pp., $20.00. The Williams & Wilkins Co., Baltimore, Md.

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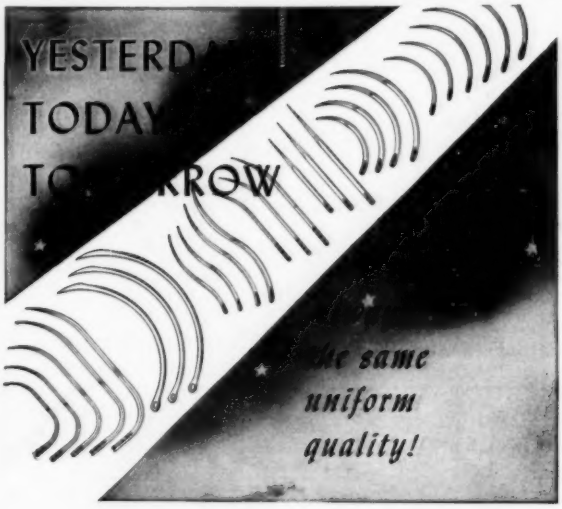

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